The American Journal of Medicine

The American Journal of Medicine

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The American Journal of Medicine

Vol. XX APRIL, 1956 No. 4

Editorial

Serologic Reactions in Rheumatoid Arthritis. WALLACE EPSTEIN AND CHARLES RAGAN 487

Clinical Studies

The Hemagglutination Test for Rheumatoid Arthritis. III. Clinical Evaluation of the Sheep Erythrocyte Agglutination (S.E.A.) Test and the Gamma Globulin (FII) Tests

ABRAHAM S. JACOBSON, WILLIAM H. KAMMERER, JULIUS WOLF, WALLACE V. EPSTEIN AND GEORGE HELLER 490

This paper summarizes a large clinical experience with two serologic tests for rheumatoid arthritis, the sheep erythrocyte agglutination (S.E.A.) procedure and the gamma globulin or fraction II (FII) procedure. These tests are positive in about 65 per cent of patients with overt rheumatoid arthritis, the incidence of positive results increasing with duration, progression and activity of the disease process. Rheumatoid spondylitis and arthritis with psoriasis give 10 to 15 per cent positive results; in other forms of arthritis the incidence of high titers is negligible. Currently available methods of treatment of rheumatoid arthritis have little influence on these serologic reactions. The mechanisms involved in these tests, still a matter of speculation, are discussed interestingly.

Agglutination and Inhibition by Serum Globulin in the Sensitized Sheep Cell Agglutination Reaction in Rheumatoid Arthritis

MORRIS ZIFF, PATRICIA BROWN, JOSEPH LOSPALLUTO, JACQUES BADIN AND CURRIER MCEWEN 500

Efforts to develop a sensitive and specific laboratory test for rheumatoid arthritis, a desideratum in view of not infrequent difficulties in clinical diagnosis, continue unabated. The present study is one of the most interesting and apparently successful such attempts, which takes into account the inhibiting effect on the sensitized sheep cell agglutination reaction of a factor or factors present in rheumatoid and non-rheumatoid serums. Removal of this inhibitor from rheumatoid serums enhances the sensitivity of the agglutination test without sacrifice of specificity. Of interest also are the results of this modified test, and of certain variations in juvenile rheumatoid arthritis, rheumatoid spondylitis, psoriatic arthropathy and other controversial disorders.

Synovial Specimens Obtained by Knee Joint Punch Biopsy. Histologic Study in Joint Diseases

HENRY A. ZEVELY, A. JAMES FRENCH, WILLIAM M. MIKKELSEN AND IVAN F. DUFF 510

Punch biopsy of the synovium of the knee joint offers a method of examination which, like all biopsy procedures, is limited in diagnostic scope and reliability but nevertheless often yields useful

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NEW CONCEPT IN URINE-SUGAR TESTING

CLINISTIX
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specific enzyme test for urine glucose

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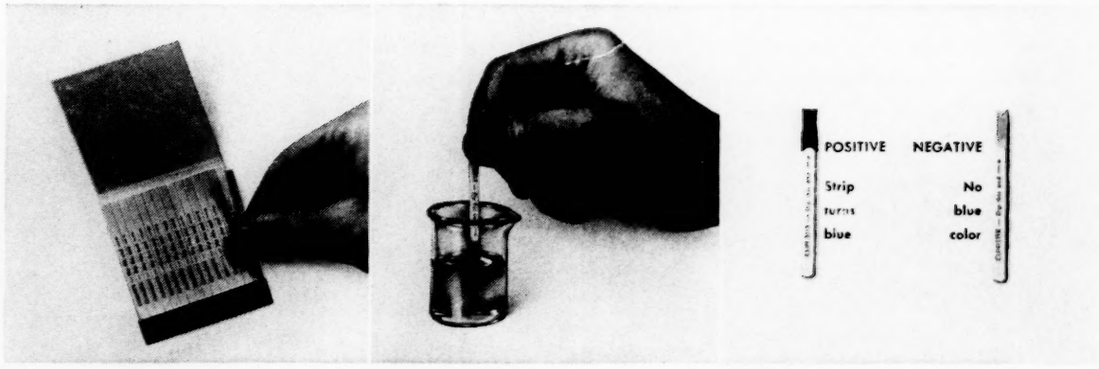

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VOLUME TWENTY NUMBER FOUR

and sometimes decisive information in obscure arthritides. This is amply demonstrated in the present factual report of a fairly large and discriminating experience. The method deserves more extensive use.

A Clinical, Physiologic and Biochemical Study of Patients with Malignant Carcinoid (Argentaffinoma)

ALBERT SJOERDSMA, HERBERT WEISSBACH AND SIDNEY UDENFRIEND 520

The subject of malignant carcinoid has recently attracted widespread interest because of the discovery that such tumors secrete serotonin (5-hydroxytryptamine) and in excessive amounts, and that the extraordinary associated clinical features are largely attributable to excessive serotoninemia. The present elegant study describes six patients with the malignant carcinoid syndrome (the case histories make fascinating reading), indicates that the diagnosis can now be made by chemical detection of a serotonin metabolite (5-hydroxyindole acetic acid) in the urine in greater than normal amounts, and goes on to consider the metabolic relations of serotonin to dietary tryptophan. Many other points of interest are made.

Essential Cryoglobulinaemia. Review of the Literature and Report of a Case Treated with ACTH and Cortisone

ROBERT VOLPÉ, ALAN BRUCE-ROBERTSON, A. ALMON FLETCHER AND W. BRUCE CHARLES 533

The authors give a detailed clinical description of the course of a patient with essential cryoglobulinemia, together with observations on the characteristics of the cryoglobulin present, and indicate how the properties of the protein in question explain the clinical manifestations, at least in part. They find it necessary, however, to postulate an associated tissue sensitization reaction to the precipitated cryoglobulin for plausible reasons explained in the text. The exposition as a whole, taken together with an analysis of the literature, makes for a most interesting paper, illustrating once again how much can be learned from intensive and intelligently directed study of a single case.

Insulin-Zinc Suspensions. Further Studies, with Emphasis on Lente Insulin

JOSEPH L. IZZO 554

The effects of lente insulin and NPH insulin were compared in carefully controlled studies in six patients with unstable diabetes. The daily distribution of activity of these two insulins was similar, although the action of lente insulin was found to be slightly slower than that of NPH insulin. The author suggests that by making available appropriate premixtures of amorphous and crystalline insulin-zinc suspensions, it might be possible to establish a small group of preparations which would meet most if not all the varied requirements of patients with unstable as well as stable diabetes.

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Reviews

Cryo- and Macroglobulinemia. Electrophoretic, Ultracentrifugal and Clinical Studies
IAN R. MACKAY, NILS ERIKSEN, ARNO G. MOTULSKY AND WADE VOLWILER 564

The origin and nature of cryoproteins and macroglobulins and the clinical symptomatology caused by their presence in the blood in appreciable quantities, are subjects which have aroused a great deal of recent interest and speculation. The present study describes five such cases and then brings together, in critically integrative fashion, the available information on this most intriguing subject. Cryoglobulinemia and macroglobulinemia are probably not primary disorders but reflect disturbances in protein formation secondary to some underlying disease, in the first instance usually multiple myeloma. Their presence in quantity, however, of itself evokes diverse symptoms and signs which may present puzzling diagnostic problems. This review is therefore well worth thoughtful reading from the clinical as well as the physicochemical point of view.

Monocytic Leukemia . . . . . CHARLES M. SINN AND FREDERICK W. DICK 588

There has been a long debate as to whether monocytic leukemia occurs as a discrete entity at all and, if so, how it may be distinguished from other forms of leukemia at least in its clinical manifestations. The present study performs a useful service in clarifying this situation in respect to chronic monocytic leukemia of which fourteen acceptable cases are summarized from the literature and eight new cases are described. The authors make clear that, unlike acute monocytic and other leukemias, the initial phases of chronic monocytic leukemia usually present with nondescript anemia and other cytopenias, associated with a non-diagnostic bone marrow and a variety of manifestations of increased susceptibility to infection. The typical blood and bone marrow abnormalities of monocytic leukemia usually appear only in the terminal phases of the disorder. This leads to a provocative discussion of the meaning of the "preleukemic" phase of leukemias in general, a phenomenon which is by no means rare and is certainly an intriguing aspect of the leukemia problem.

Seminar on Allergy

Diagnostic Methods for Allergic Diseases . . . . . WILLIAM B. SHERMAN 603

Dr. Sherman briefly considers the importance of history taking in detecting specific allergenic factors in disease and, in greater detail, the skin test technics. The usefulness and limitations of allergic skin reactions are carefully defined, with many practical pointers to guide the unwary. Then follows an appraisal of elimination diets and the food diary as aids to confirmation of the presence of food allergy. The discussion closes with a consideration of other diagnostic technics employed less extensively. The whole makes for a sober evaluation and leaves one with the feeling that this important segment of the physician's activity may be said to have been more sinned against than sinning.

Clinico-pathologic Conference

Recurrent Burning Retrosternal Pain 612

Clinico-pathologic Conference (Washington University School of Medicine).

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CONTENTS continued–April 1956

VOLUME TWENTY NUMBER FOUR

Case Reports

Cryoproteinemia: An Immunologic Phenomenon? Electrophoretic Analysis of Serum Proteins of a Patient with Cold Allergy
Z. T. WIRTSCHAFTER, D. W. WILLIAMS AND E. C. GAULDEN 624

Cryoproteins continue to intrigue the clinician and the chemist. The present study is of unusual interest because it involves separation of a cryoprotein from the blood of a patient with all the clinical manifestations of cold allergy, and nothing else, with enlightening observations on the properties of the cryoprotein so obtained. Administration of cortisone resulted in disappearance of the cryoprotein from the blood and, with this, remission of the "cold allergy."

Studies on a Patient with Severe Hyperinsulinism Treated with Cortisone for Three Years . . . . . HERBERT GERSHBERG AND ELAINE P. RALLI 631

An unusually interesting case of well studied hyperinsulinism responding for three years to treatment with cortisone. Among the significant points brought out clearly in this report is the relationship between vasomotor lability and hypoglycemia, a relationship not generally appreciated.

Pericardial Effusion in Generalized Scleroderma . . . . . JAY I. MELTZER 638

Two cases of generalized scleroderma with substantial pericardial effusion, presumably related to the underlying disease, are described.

Infectious Mononucleosis of the Central Nervous System. Demonstration of Atypical Lymphocytes in the Cerebrospinal Fluid
LEO E. HOLLISTER, GEORGE H. HOUCK AND WILLIAM A. DUNLAP 643

A report of unusual interest, describing a case of infectious mononucleosis of the central nervous system in which a high proportion of atypical lymphocytes was found in the spinal fluid, an observation recorded, apparently, for the first time. No atypical lymphocytes could ever be detected in the blood. Heterophile antibodies characteristic of infectious mononucleosis were demonstrated in significant titer in blood and spinal fluid.

Dextroposition of the Heart Simulating Congenital Dextrocardia
WERNER J. HOLLENDONNER AND BERNARD H. PASTOR 647

An interesting case.

Erratum: In the December 1955 issue of The American Journal of Medicine, the article entitled "Spontaneous Hypopotassemia, Hypomagnesemia, Alkalosis and Tetany Due to Hypersecretion of Corticosterone-like Mineral-ocorticoid" by Drs. Ivan J. Mader and Lloyd T. Iseri, the first paragraph of the Addendum should begin: On February 28, 1955, the patient was operated upon and the left adrenal gland, containing a cortical tumor, 2.5 cm. in diameter and 6.5 gm. in weight, was removed.

Advertising Index on 3rd Cover

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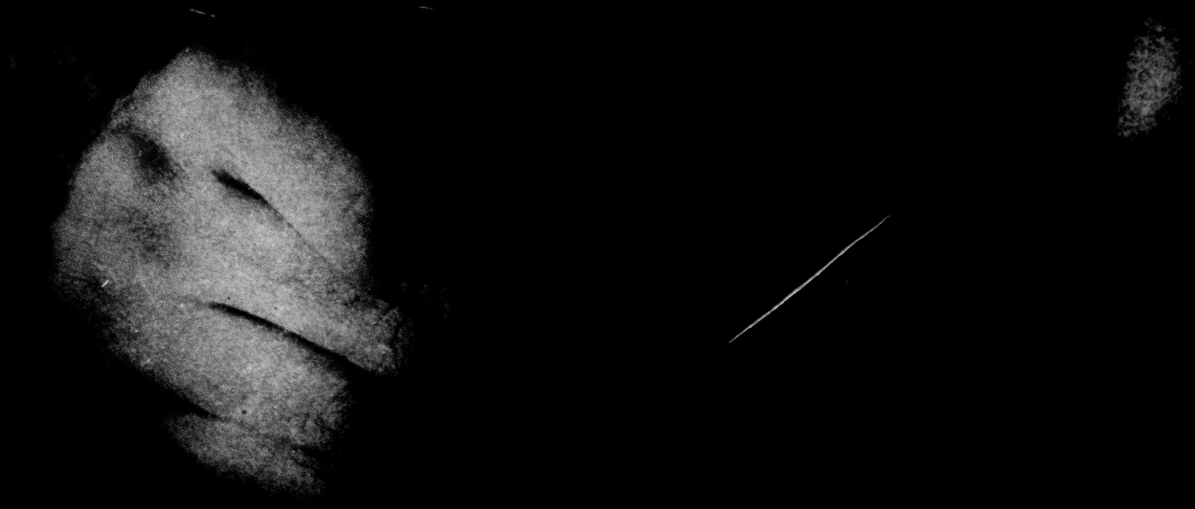

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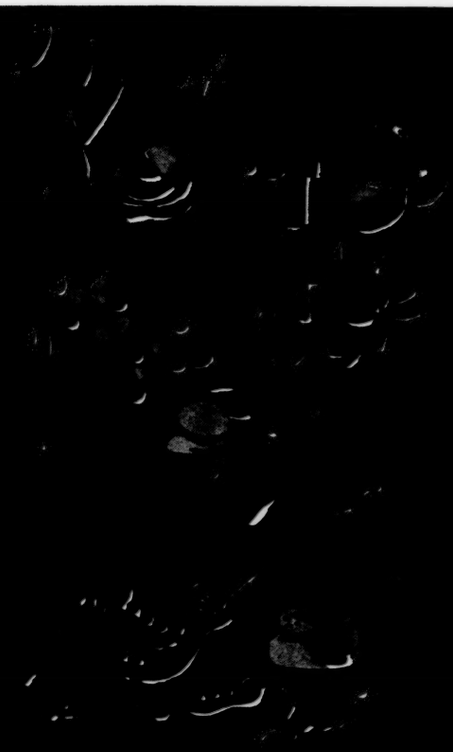

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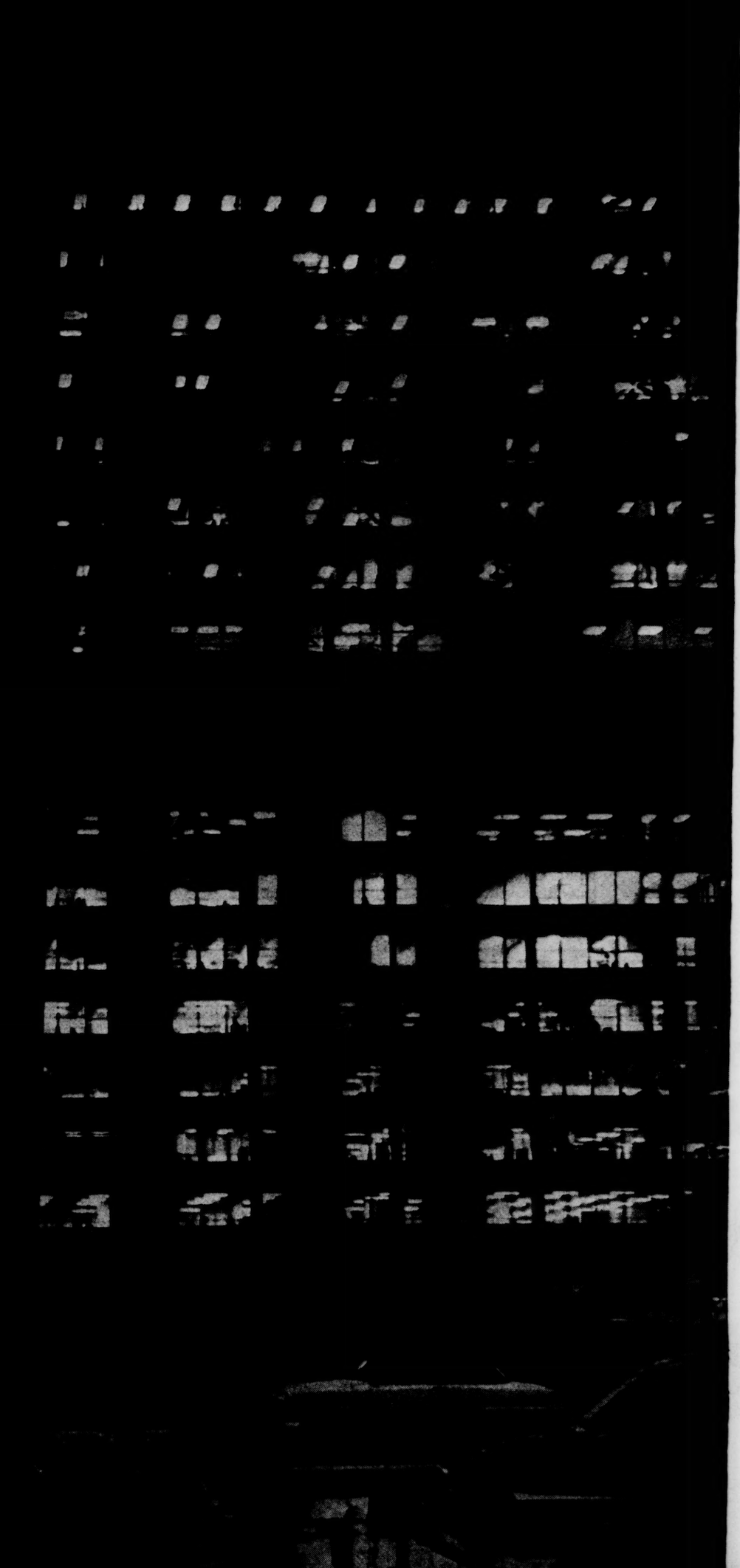

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[1]January, H. L. et al: Clinical experience with tetracycline. *Antibiotics Annual* 1954-55, p. 625.

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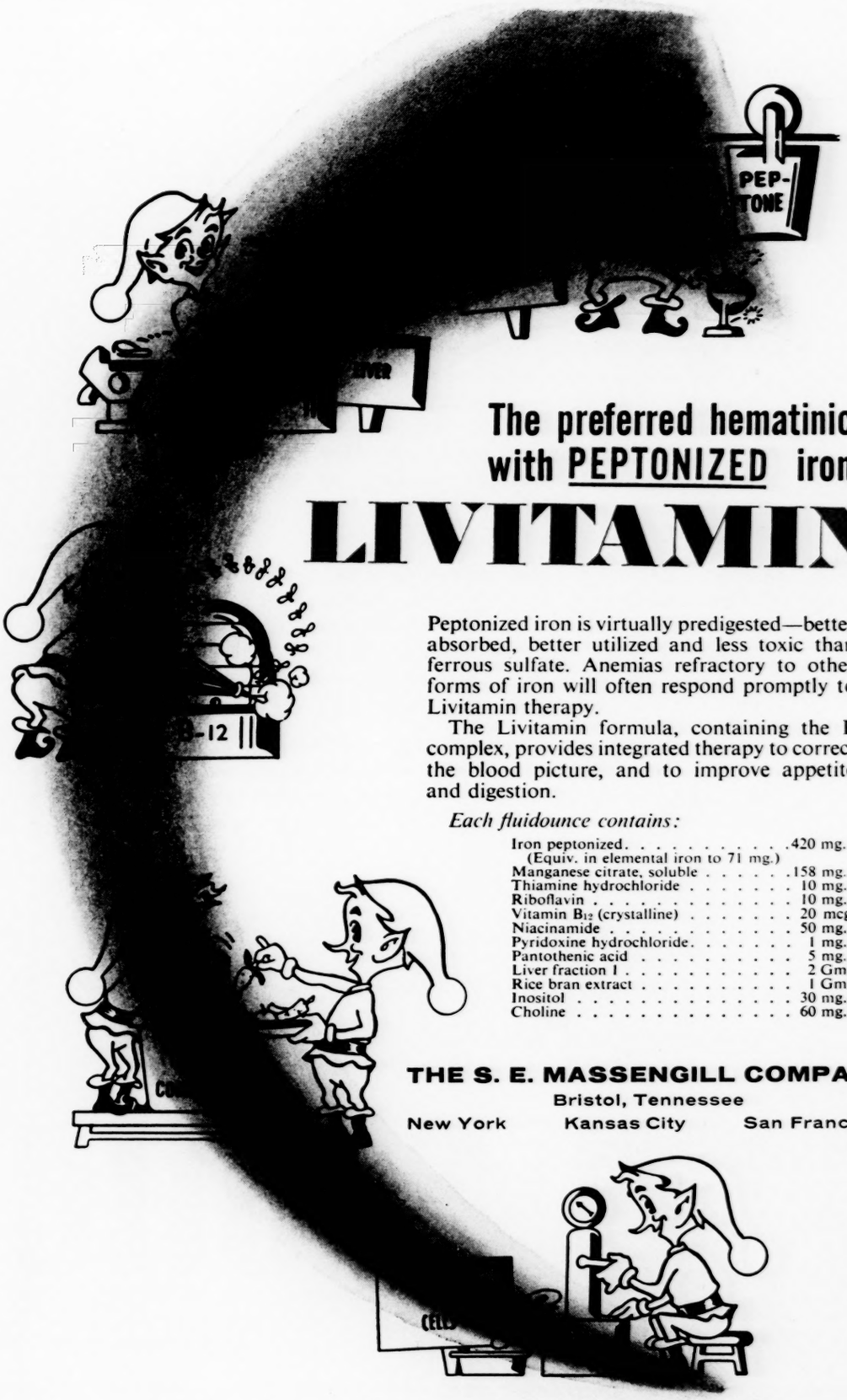

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References:

1. Winsor, T.: Am. J. M. Sc. *230*:133 (Aug.) 1955. 2. Grimson, K. S.: J.A.M.A. *158*:359 (June 4) 1955. 3. Grimson, K. S., Tarazi, A. K., and Frazer, J. W., Jr.: Circulation *11*:733 (May) 1955. 4. Grimson, K. S., Tarazi, A. K., and Frazer, J. W., Jr.: Angiology *6*:507 (Dec.) 1955. 5. Strawn, J. R., and Moyer, J. H.: Personal communication, 1955. 6. Maxwell, R. D. H., and Howie, T. J. G.: Brit. M. J. *2*:1189 (Nov. 12) 1955.

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4	50 mg.	50 mg.
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6	75 mg.	75 mg.
7	100 mg.	75 mg.
8	100 mg.	100 mg.

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DAY	A.M.	P.M.
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3	100 mg.	50 mg.
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		Blood pressure in 20 well controlled; reductions lasted twice as long as those induced by pentolinium. Each of 10 patients with previous experience with hexamethonium preferred Ecolid. Less difficulty with constipation; appetite improved; greater energy.	**	2
		Hypertension in 18 well controlled. Supine blood pressure reduced without tachycardia. Constipation occurred infrequently.	Supine blood pressure lowered for 12 hours or more with single oral doses of 50 to 100 mg.	3,4
		35 responded well; 14 of these became normotensive. All patients received reserpine as base therapy.	**	5
		Blood pressure of all 12 satisfactorily controlled. Systolic blood pressure lowered average of 76 mm. Diastolic blood pressure lowered [illegible]	**	6

*To date, a total of 63 investigators have reported on the use of Ecolid in more than 500 patients. They were practically unanimous in the opinion that Ecolid was highly effective. Nearly all commented on the prolonged duration of action — about 8 to 12 hours — which permitted a twice daily dosage schedule in most cases.

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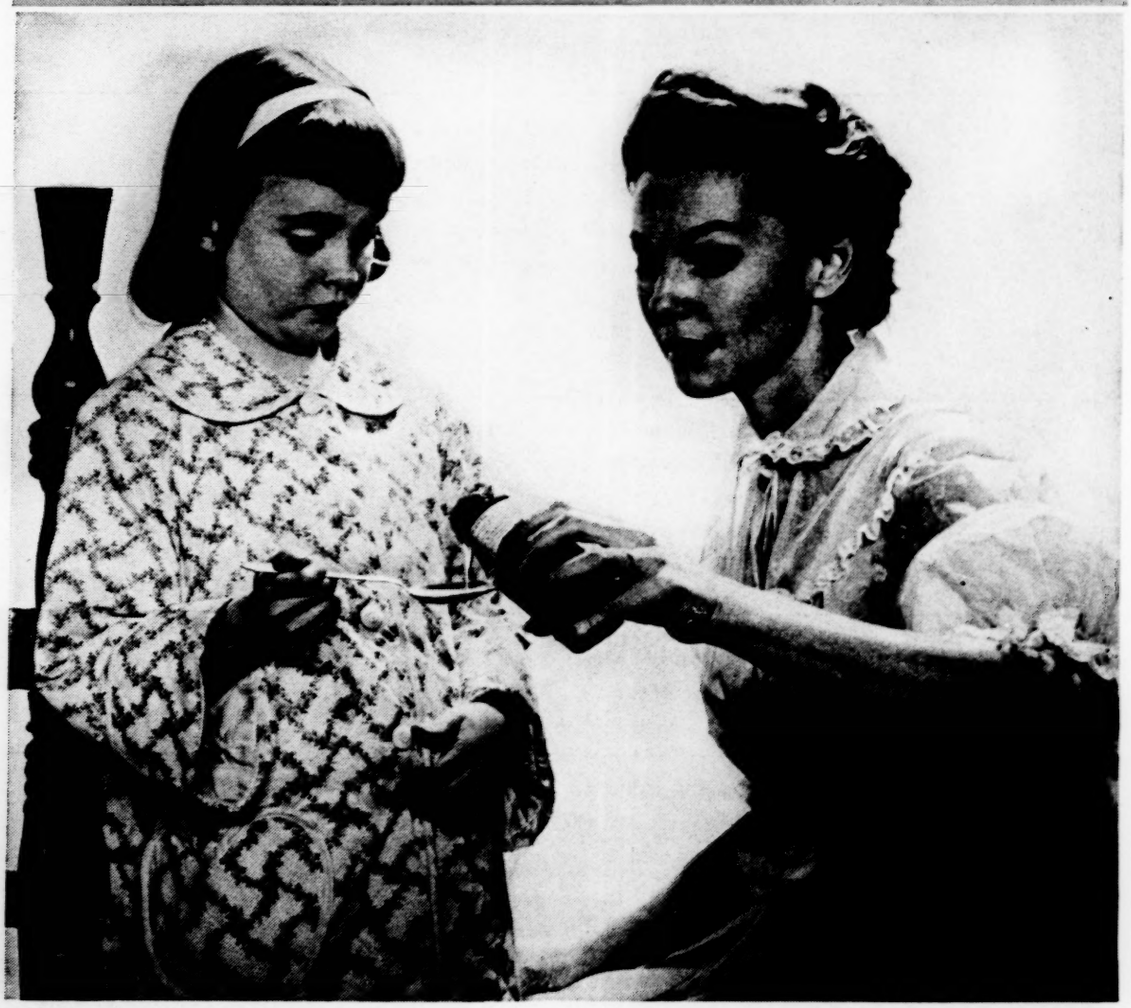


You can prescribe new CREMOMYCIN even when the whole family has diarrhea. It contains 'Sulfasuxidine' and neomycin. Both are sparingly absorbed – thus they are virtually nontoxic and their action is concentrated in the intestine.[1] Pectin and kaolin adsorb toxins and soothe the mucosa.

Supplied: 8-oz. bottles, each fl. oz. (30 cc.) containing 3.0 *Gm.* 'Sulfasuxidine,' 300 *mg.* of neomycin sulfate, 0.3 *Gm.* of pectin and 3.0 *Gm.* of kaolin.

Reference: 1. Poth, E. J., J.A.M.A. 153:1516 (Dec. 26) 1953.

SHARP & DOHME

Philadelphia 1, Pa.
DIVISION OF MERCK & CO., INC.

new...paired piperidol action
for functional gastrointestinal complaints

rapid
visceral eutonic, Dactil*

prolonged
cholinolytic, Piptal†

relief throughout the G. I. tract

Tridal

TRIDAL permits more comprehensive control of gastrointestinal complaints by providing the combined benefits of two piperidols. The local action of Dactil* works immediately to give **rapid** relief of gastrointestinal pain and spasm; the potent cholinolytic Piptal† reinforces relief and provides **prolonged** normalization of secretion and motility.

TRIDAL is singularly free from urinary retention, constipation, dry mouth, blurred vision.

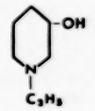
dosage: One TRIDAL Tablet two or three times a day and at bedtime. Unless rapidly swallowed with water, TRIDAL will produce some lingual anesthesia.

Each TRIDAL Tablet contains 50 mg. of Dactil and 5 mg. of Piptal. Bottles of 50 compressed, uncoated tablets.

*Dactil (the *only* brand of N-ethyl-3-piperidyl diphenylacetate hydrochloride): the piperidol to prescribe alone when no interference with digestive secretion is desired.

†Piptal (the *only* brand of N-ethyl-3-piperidyl-benzilate methobromide): the piperidol to prescribe alone when peptic ulcer is known to be present and normalization of secretion as well as motility is desired.

Lakeside Laboratories, Inc PIONEERS IN PIPERIDOLS MILWAUKEE 1, WISCONSIN



93193

megaloblastic anemia

one of the many anemias which can be effectively treated with

PERIHEMIN*

Hematinic Lederle

Nine out of 10 of all treatable anemias respond to PERIHEMIN. Its potent formula includes every known hemopoietic agent, including Purified Intrinsic Factor Concentrate. With this single product, you provide complete anemia therapy in a form convenient for the patient.

Dosage: one capsule, t.i.d.

Each capsule contains:

Vitamin B_{12} with Intrinsic Factor Concentrate	½ U.S.P. Oral Unit
Vitamin B_{12} (additional)	5 mcgm.
Ferrous Sulfate (Exsiccated)	192 mg.
Folic Acid	0.85 mg.
Ascorbic Acid (C)	50 mg.
Insoluble Liver Fraction	50 mg.

PERIHEMIN JR *Capsules*, for children, are approximately one-quarter the potency of this formula.

dry filled sealed capsules (a Lederle exclusive!) for more rapid and complete absorption!

LEDERLE LABORATORIES DIVISION *AMERICAN CYANAMID COMPANY* PEARL RIVER, NEW YORK

*REG. U.S. PAT. OFF.

NEW

for urinary infections

AZO GANTRISIN

pain relief plus antibacterial therapy

In cystitis, prostatitis, urethritis and other urinary infections, Azo Gantrisin 'Roche' provides:

1. Wide antibacterial spectrum -- effective against a wide range of microorganisms.
2. Relief of pain, burning, frequency and discomfort; soothing effect on mucosa.
3. High plasma levels -- important in hematogenous urinary tract infections.

Each Azo Gantrisin tablet contains 0.5 Gm Gantrisin 'Roche' plus 50 mg phenylazo-diamino-pyridine HCl (for local pain relief).

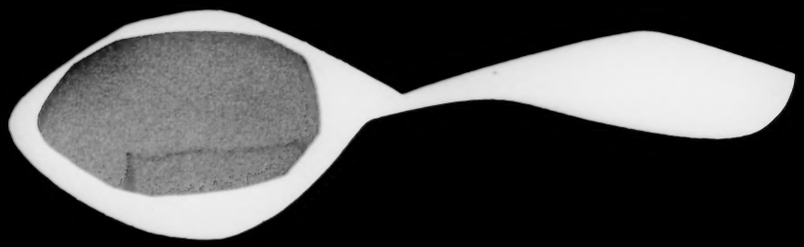
Gantrisin® -- brand of sulfisoxazole

Gentle is the word for Noludar—

Mild, yet positive in action, Noludar 'Roche' is especially suited for the tense patient who needs to relax and remain clear-headed - or for the insomniac who wants a refreshing night's sleep without hangover. Not a barbiturate, not habit-forming. Tablets, 50 and 200 mg; elixir, 50 mg per teasp.

ROCHE
Original Research
in Medicine and Chemistry

Noludar®—brand of methyprylon (3,3-diethyl-5-methyl-2,4-piperidinedione)



swallowed with a smile

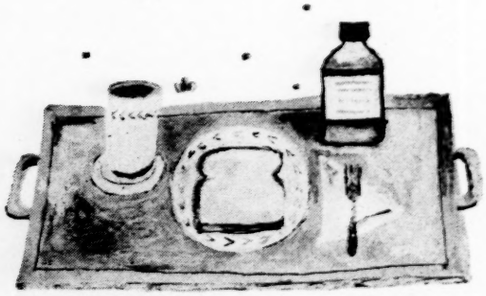
liquid vitamin supplement

faster
paced...
better
taste

new broad-spectrum

Tetrabon*

BRAND OF TETRACYCLINE



homogenized mixture

125 mg. tetracycline per 5 cc. teaspoonful. *Bottles of 2 fl. oz. and 1 pint, packaged ready to use.*

READY TO USE No reconstitution required.

READILY ACCEPTED Unusual, delicious fruit flavors.

RAPIDLY ABSORBED Fine particle dispersion—therapeutic blood levels within one hour.

RAPIDLY EFFECTIVE Fast, trouble-free tetracycline for control of the widest range of infections.

also available: vitamin-fortified TETRABON SF† (brand of tetracycline hydrochloride with vitamins) *homogenized mixture*: 125 mg. tetracycline per 5 cc. teaspoonful, plus vitamins of the B complex, C and K recommended for nutritional support in the stress of prolonged infection.

Bottles of 2 fl. oz., packaged ready to use.

*Trademark †Trademark for Pfizer-originated, vitamin-fortified antibiotics

Pfizer PFIZER LABORATORIES, *Division, Chas. Pfizer & Co., Inc.*, Brooklyn 6, N. Y.

Upjohn

Relax
the nervous,
tense,
emotionally unstable:

Reserpoid *(Pure crystalline alkaloid)*

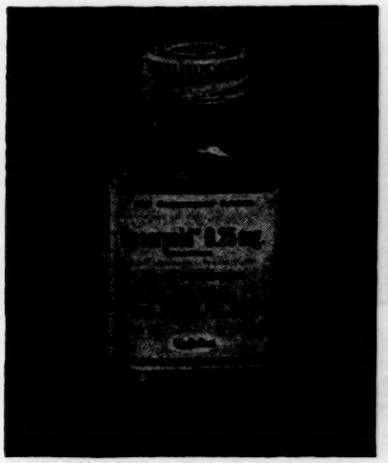
TRADEMARK FOR THE UPJOHN BRAND OF RESERPINE

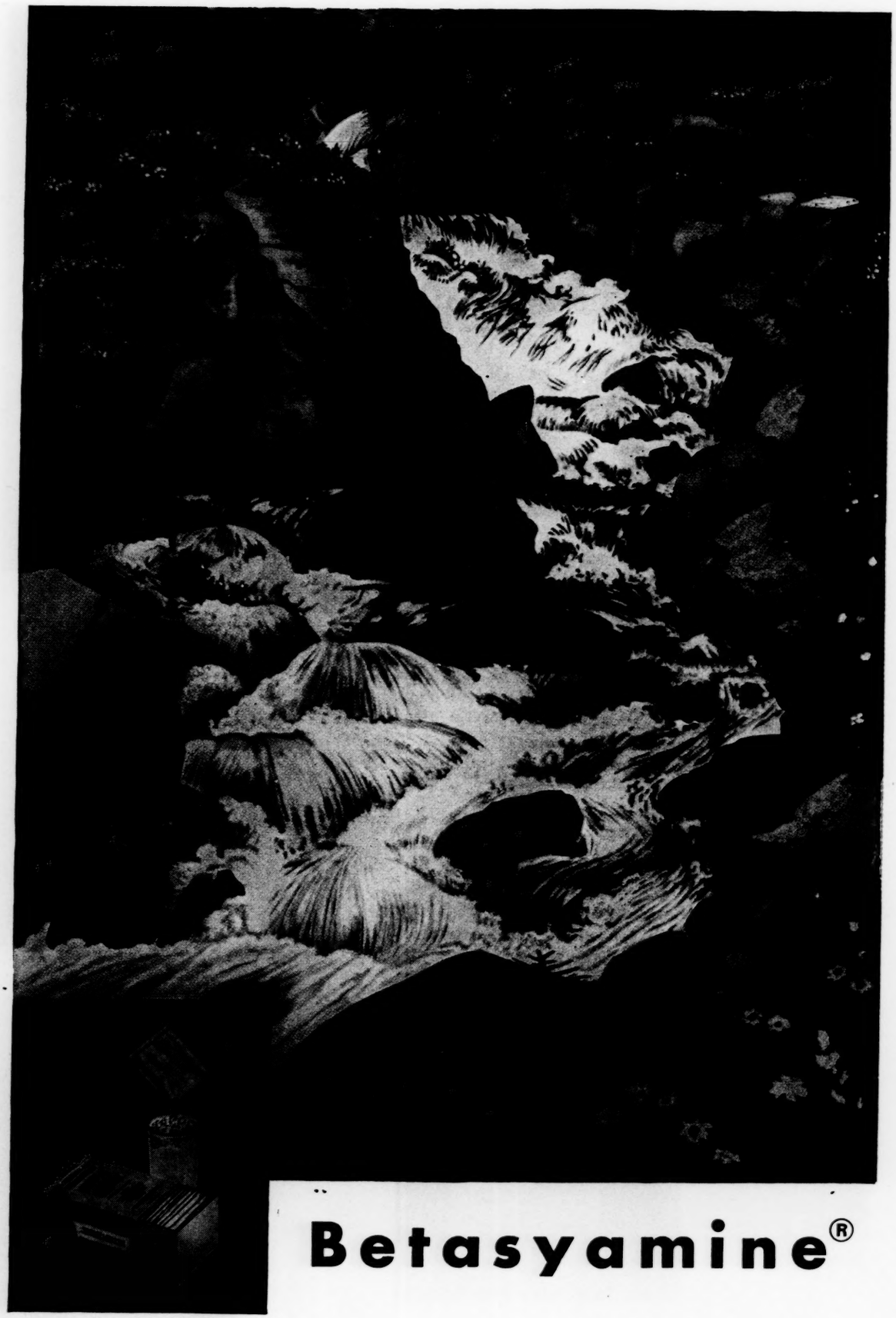
Each tablet contains:
Reserpine . . . . . 0.1 mg.
or 0.25 mg.
or 1.0 mg.
or 4.0 mg.

The elixir contains:
Reserpine . . . . . 0.25 mg.
per 5 cc. teaspoonful

Supplied:
Scored tablets
0.1 and 0.25 mg. in bottles of 100 and 500
1.0 and 4.0 mg. in bottles of 100
Elixir in pint bottles

The Upjohn Company, Kalamazoo, Michigan





Betasyamine®

Betasyamine® . . . for a fresh outlook . . .
in your "well" patients who feel sick.

These are your patients: Prominent in your practice are those patients not demonstrably ill, but always below par — mentally, physically, emotionally. These are your "problem patients." How to treat them? Hirsch[1] has furnished a clue. He points out an ever present condition:

". . . a depletion of energy up to or beyond the body's ability to spring back."

The fatigue syndrome is often linked with subnormal muscle and nerve phosphocreatine readings.[2] Betasyamine, containing betaine and glycocyamine, precursors of phosphocreatine, steps up these levels to normal, thus tending to restore and maintain the dynamic energy balance. Containing no unphysiologic sedative or stimulant drug, Betasyamine offers promise wherever increased burdens and strains have undermined the energy reserve.

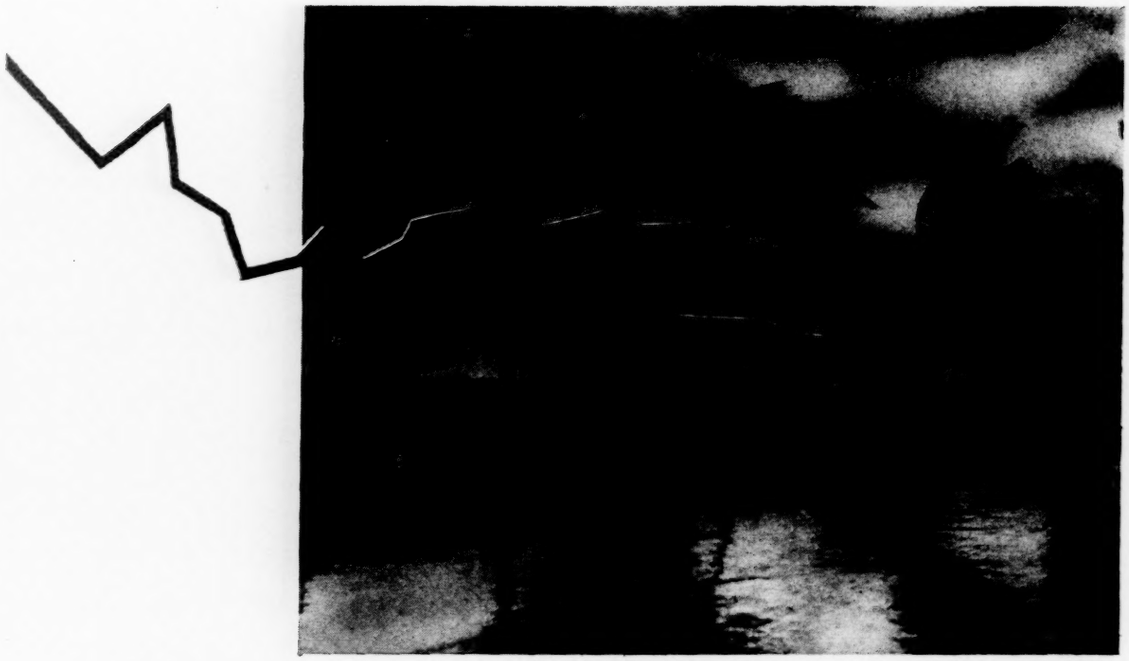
Fatigue and depression frequently result from the rigid therapeutic and dietary programs required in diabetes allergy and obesity management. Difficult postsurgical and obstetrical periods — prolonged infectious sieges — keep patients discouraged and debilitated — unable to "spring back." Betasyamine, included in the recovery programs of these and many other conditions characterized by low energy states, provides welcome relief from depressing and taxing exhaustion. Betasyamine helps to create a new mood . . . for a fresh outlook.

Average Dosage: 3 Effervescent Packets; 3 tablespoonfuls Emulsion; or 15 Tablets: (three times daily at mealtimes).
Supplied: Effervescent Packets (new) — 24's; Emulsion — 16 fl.oz.; Tablets — 200's.

1. Hirsch, S.: New York J. Med. *55:1170* (April 15) 1955. 2. Dixon, H. H.; and others: West. J. Surg. *60:327* (July) 1952).

Amino Products Division • International Minerals & Chemical Corporation • Chicago • San Francisco

for a fresh outlook



when neuritis strikes

how long need it last?

Instead of enduring long weeks of pain and disability, your patients with inflammatory radiculitis (of non-traumatic or non-mechanical origin) can usually be quickly relieved with Protamide. When used promptly—within a few days after onset of pain—complete recovery can be expected in just a few days.

Published studies* and experience in many thousands of cases treated in private practice demonstrate these advantages—even in types of neuritis intractable to older therapies. You can duplicate these results in your practice. Keep Protamide on hand for use at the patient's first visit.

Available at pharmacies and supply houses—
boxes of ten 1.3 cc. ampuls.

use **PROTAMIDE®** *promptly*

...one ampul daily, intramuscularly

Sherman Laboratories Detroit 11, Michigan

*A portfolio of all published studies will be sent on request

All the benefits of prednisone and prednisolone plus positive antacid action to minimize gastric distress

Multiple Compressed Tablets of 'Co-Deltra' and 'Co-Hydeltra' are designed to help the physician cope with the problem of gastric distress which might otherwise become an obstacle to therapy with the newer steroids prednisone and prednisolone. Each Multiple Compressed Tablet is specifically formulated as a "tablet within a tablet" to provide stability and to release in sequence antacid and anti-inflammatory components.

NEW

'Co-Hydeltra'

Prednisolone Buffered

MULTIPLE COMPRESSED TABLETS

and

'Co-Deltra'

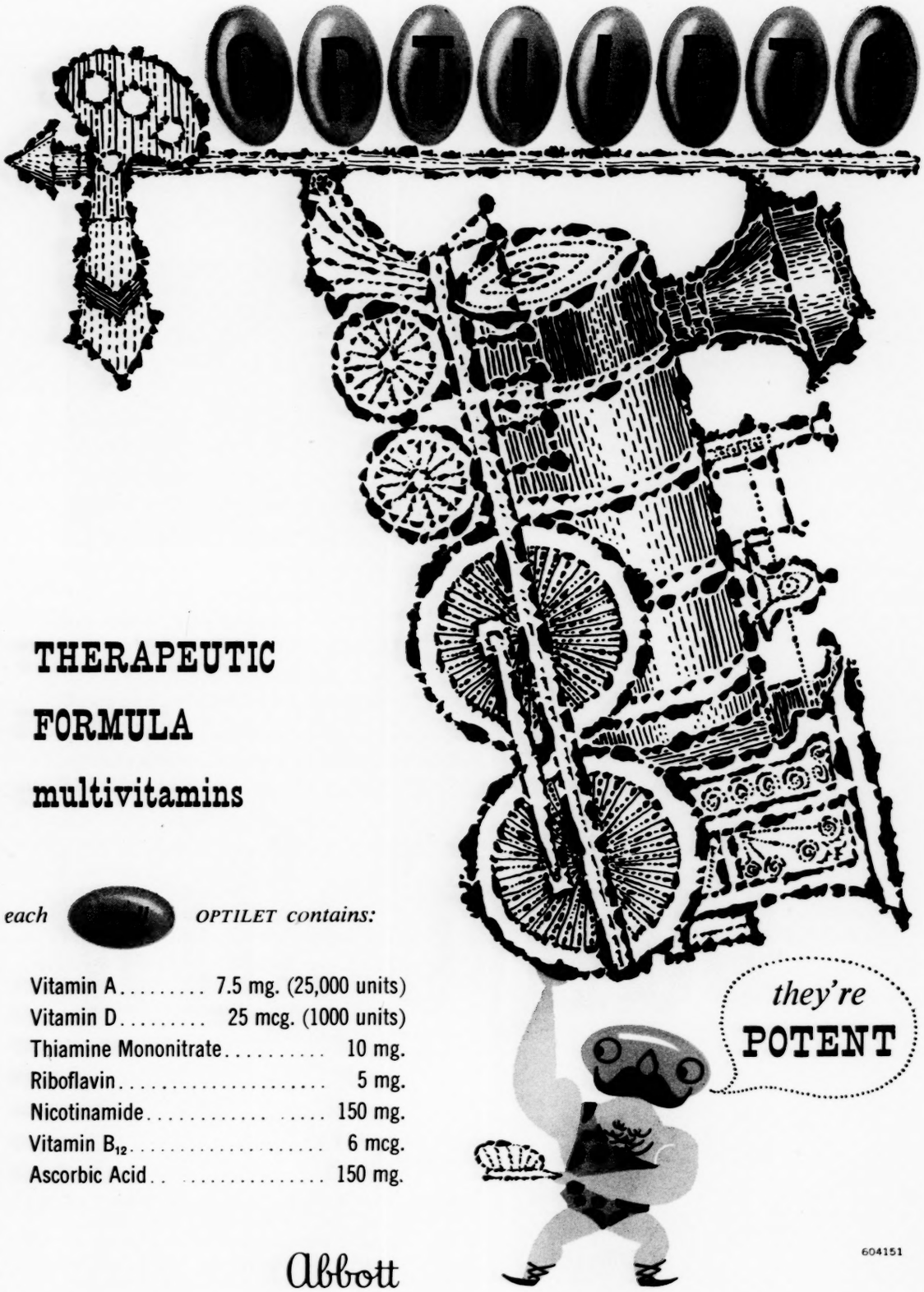
Prednisone Buffered

Supplied: Multiple Compressed Tablets of 'Co-Deltra' and 'Co-Hydeltra', each containing 5 mg. prednisone or prednisolone, 300 mg. of dried aluminum hydroxide gel, U.S.P., and 50 mg. of magnesium trisilicate, U.S.P., bottles of 30 tablets.

SHARP & DOHME

Philadelphia 1, Pa.
DIVISION OF MERCK & CO., INC.

'Co-Deltra' and 'Co-Hydeltra' are the trademarks of MERCK & CO., INC.



THERAPEUTIC FORMULA multivitamins

each *OPTILET contains:*

Vitamin A	7.5 mg. (25,000 units)
Vitamin D	25 mcg. (1000 units)
Thiamine Mononitrate	10 mg.
Riboflavin	5 mg.
Nicotinamide	150 mg.
Vitamin B_{12}	6 mcg.
Ascorbic Acid	150 mg.

Abbott

604151


XYLOCAINE
HYDROCHLORIDE 0.5%
with EPINEPHRINE
2cc
ASTRA
to try it .....
XYLOCAINE
HYDROCHLORIDE
with EPINEPHRINE

fastest acting local anesthetic— as safe as it is effective

How safe is Xylocaine? In five years, over 500,000,000 injections of Xylocaine HCl Solution have been given for local anesthesia. "The apparent clinical safety of Xylocaine is gratifying, for without this quality, its additional properties would not warrant an enthusiastic report. Nor would safety alone call for a high recommendation unless additional desirable properties were to be found. The truth of the matter is, however, that Xylocaine approaches the ideal drug more closely than any other local anesthetic agent we have today."*

How effective is Xylocaine? Xylocaine HCl Solution produces more rapid, complete, and deeper anesthesia than other local anesthetics used in equivalent doses. By infiltration, Xylocaine gives a wide area of analgesia, and surrounding tissues are also anesthetized. The long duration of Xylocaine action reduces the need for additional injections. At the same time, it assures greater comfort to your patients for a longer period—often when they need it most.

How does Xylocaine fit into my practice? Xylocaine is the ideal agent for *local infiltration anesthesia* because it is safe, fast acting and of long duration. It is used routinely in daily practice for countless minor surgical procedures such as closure of lacerations, removal of cysts, moles and warts; treatment of abscesses; and in the reduction of fractures.

....is to use

XYLOCAINE®

It has also become the choice of many physicians for *therapeutic interruption of nerve function by temporary nerve blocks* in herpes zoster, subdeltoid bursitis, fibrositis, myalgia of shoulder muscles, periarthritis due to trauma, and painful postoperative scars. The relief of pain in these conditions at times appears to be the most important part of treatment.

The remarkable *topical anesthetic* properties of Xylocaine HCl Solution further enhance its usefulness for minor operations. Topical anesthesia can be obtained by spraying, by applying packs, by swabbing, or by instilling the solution into a cavity or on a surface.

Xylocaine HCl Solutions are available in 2 cc. ampuls, 20 cc. and 50 cc. vials in strengths of 0.5%, 1% and 2%, with or without epinephrine.

Bibliography of approximately 300 Xylocaine references upon request.

*Southworth, J. L., and Dabbs, C. H.: Xylocaine: a superior agent for conduction anesthesia, Anesth. & Analg. *32*:159 (May-June) 1953.

Astra Pharmaceutical Products, Inc., Worcester 6, Mass.

helps re-establish normal bowel function

A bulk-producing plantago derivative with thiamine which stimulates peristalsis physiologically, SIBLIN promotes the formation of stools of normal consistency for easy passage.

in a variety of clinical conditions

SIBLIN helps to restore and to maintain normal bowel function in pregnancy and during lactation, in anorectal disorders and following surgical operations involving the bowel, in chronic constipation, and in diarrhea.

SIBLIN®

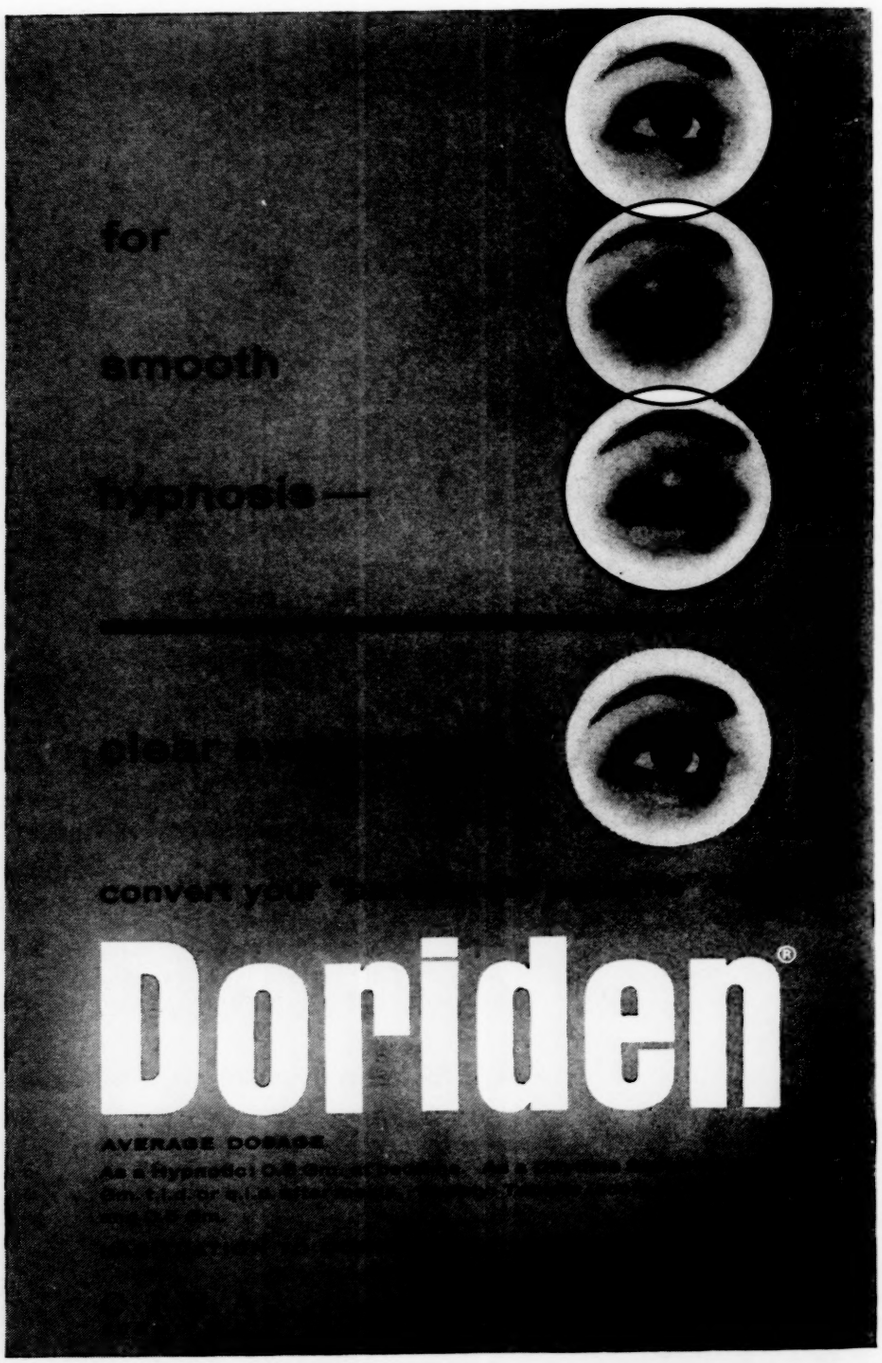
lubricant bulk with thiamine

supplied

SIBLIN (in granular form), 4-ounce and 16-ounce packages.
SIBLIN Tablets, also containing gum kayara, pectin and agar, bottles of 100 and 500.

PARKE, DAVIS & COMPANY
DETROIT, MICHIGAN

50019


for
smooth
hypnosis—
clear
convert your
Doriden®
AVERAGE DOSAGE

the Little Stroke

Progressive brain damage is most frequently associated with arteriosclerosis and capillary fragility often resulting from a variety of causes.

These include long standing hypertension; chronic renal disease whether due to glomerulonephritis, nephrosclerosis, chronic pyelonephritis. In long standing diabetics, this condition is noted in a certain percentage of patients with Kimmelstiel-Wilson Syndrome. Although the primary disease is very different in these various entities, the final pathological findings are remarkably similar, capillary fragility.

Whenever the ophthalmoscopic examination reveals hemorrhagic areas, it is reasonable to assume a comparable process is going on in the brain.

Clinical evidences of this deterioration are manifested by: reduced intellectual activity, mental confusion, impaired judgment, emotional instability, listlessness, loss of appetite, weakness and early fatigability.

What we have described are symptoms of senility. Cerebral arteriosclerosis and capillary fragility have always been a difficult problem. The addition of Hesper-C to the diet of the elderly with the above indications makes the difference in capillary strength.

HESPER-C

is the original synergistic nutritional supplement for capillary integrity and provides 100 milligrams each of Ascorbic Acid and Hesperidin concentrate.

Send for samples and reprints.

The film "CLINICAL ENZYMOLOGY" is now available for showing at medical meetings upon your request. And be sure to watch for the Med-Audiographs, a series of recorded clinical discussions.

PRODUCTS OF ORIGINAL RESEARCH

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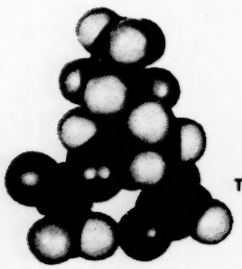
THE NATIONAL DRUG COMPANY PHILADELPHIA 44, PA.



THE MILTOWN MOLECULE

Two articles in the April 30th issue of The Journal of the AMA[1, 2] report on . . .

an entirely new type of tranquilizer with muscle relaxant action—orally effective in

ANXIETY, TENSION and MENTAL STRESS

- no autonomic side effects—well tolerated
- selectively affects the thalamus
- not related to reserpine or other tranquilizers
- not habit forming, effective within 30 minutes for a period of 6 hours
- supplied in 400 mg. tablets. Usual dose: 1 or 2 tablets—3 times a day

1. Selling, L. S.: J.A.M.A. **157:** 1594, 1955. **2.** Borrus, J. C.: J.A.M.A. **157:** 1596, 1955.

Miltown®

the original meprobamate—2-methyl-2-*n*-propyl-1,3-propanediol dicarbamate—U. S. Patent 2,724,720

DISCOVERED AND INTRODUCED by Wallace Laboratories, New Brunswick, N. J.
Literature and Samples Available On Request

Throughout the world...

use in millions of cases and reports by thousands of physicians have built confidence in

TERRAMYCIN

...well-tolerated, rapidly effective broad-spectrum antibiotic of choice.

Capsules, tablets, taste-tempting liquid forms and special preparations for parenteral, topical and ophthalmic use.

for a sound sleep tonight, a calm day tomorrow . . .

filmtab®

Nembu-Serpin®

Restful nights are added to calm days for anxiety and mild hypertension patients when you prescribe Nembu-Serpin. The 30 mg. (½ gr.) of short-acting Nembutal (Pentobarbital Calcium) in each tiny Filmtab quickly induces drowsiness at bedtime, followed by refreshing sleep. Then the 0.25 mg. of longer-acting reserpine in each Filmtab calms patients all through the following days.

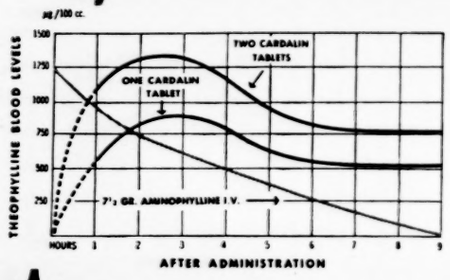
Patients experience almost immediate relief as Nembu-Serpin's sedative-tranquilizing action rapidly takes effect. Then their sense of well-being increases during the following few days. Nembu-Serpin avoids prolonged waiting for a cumulative response to reserpine.

Small dosages add safety, simplicity, economy for patients. Dosage schedules are simple, medication economical: just one Nembu-Serpin Filmtab at bedtime will calm the worries of most anxiety patients.
Nembu-Serpin Filmtabs—in bottles of 100 and 500. Abbott

® *Film-sealed tablets*

604161

It takes therapeutic blood levels



Sustained theophylline blood levels were higher with 1 or 2 oral Cardalin tablets than with 7½ gr. of aminophylline I.V.

(Adapted from Bickerman, H. A., et al.: Ann. Allergy 11:301, 1953, and Truitt, E. B., Jr., et al.: J. Pharmacol. & Exper. Therap. 100:309, 1950.)

to produce therapeutic results

When the asthmatic gasps for air...

Cardalin

Excellent increases in vital capacity and maximum breathing capacity.

Bickerman, H. A., et al.: Ann. Allergy 11:309, 1953.

Produces productive coughing following bronchodilatation.

Barach, A. L., et al.: Dis. of Chest 23:121, 1953.

Continued relief of paroxysms of bronchospasm.

Segal, M. S., et al.: Quart. Rev. Allergy & Applied Immunol. 6:399, 1952.

Each Cardalin tablet supplies:

Aminophylline .... 5.0 gr.
Aluminum Hydroxide..... 2.5 gr.
Ethyl Aminobenzoate..... 0.5 gr.

Bottles of 50, 100, 500, 1000.

Also available: Cardalin-Phen, also containing ¼ gr. phenobarbital per tablet.

To serve your patients today—Call your pharmacist for any additional product information you may need to help you prescribe Cardalin.

IRWIN, NEISLER & COMPANY • DECATUR, ILLINOIS

in pregnancy... a good nutritional start

NATABEC® KAPSEALS®

vitamin-mineral combination

Prescribed early in pregnancy, NATABEC Kapseals get your patients off to a good nutritional start—help keep vitamin-mineral intake abreast of increased nutritional needs. NATABEC Kapseals provide iron and calcium, as well as important vitamins in a formulation expressly designed to protect the health of both mother and child.

DOSAGE: As a dietary supplement during pregnancy and lactation, one or more Kapseals daily. Available in bottles of 100 and 1,000.

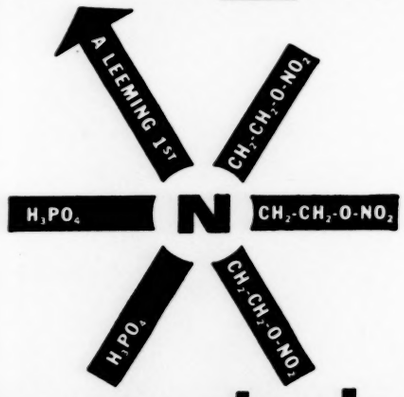
PARKE, DAVIS & COMPANY DETROIT, MICHIGAN

Metamine®

triethanolamine trinitrate biphosphate, LEEMING, tablets 2 mg. Bottles of 50 and 500
Dose: 1 or 2 tablets after each meal and at bedtime.

smallest dose lowest toxicity unique amino nitrate

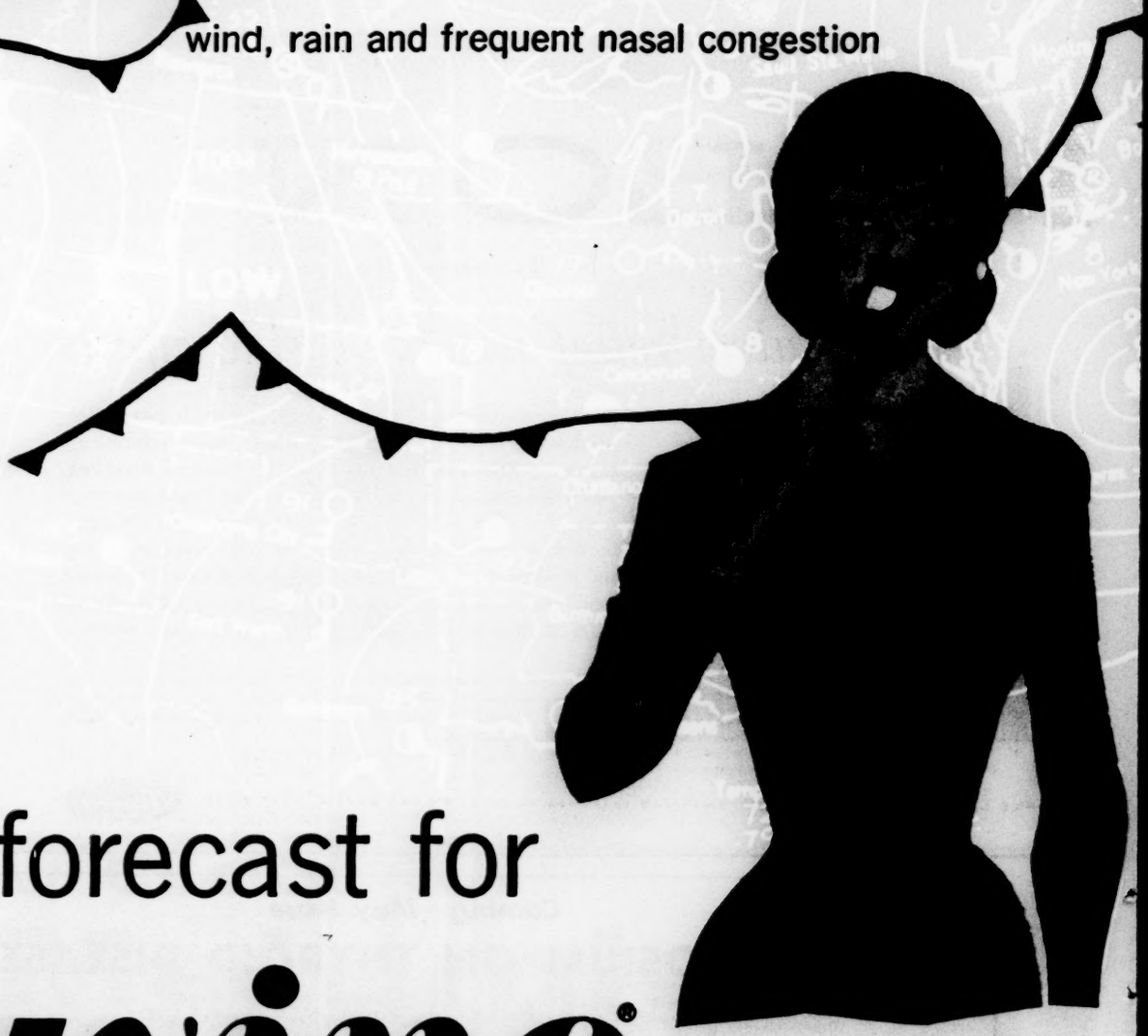
protects
8 out of 10
patients
against angina pectoris



Thos. Leeming & Co., Inc., 155 East 44th Street, New York 17, N. Y.

forecast:

wind, rain and frequent nasal congestion



a forecast for Tyzine®

brand of tetrahydrozoline hydrochloride

a "superior"[1-3] nasal decongestant

providing nasal patency in minutes (almost immediately after instillation) for hours (up to 6 hours with a single dose) without rebound engorgement...

odorless, tasteless and free of sting, burn or irritation

"In addition ... [TYZINE] shortened the usual course of each infection, and, also either prevented or aborted complications..."[1]

supplied:

TYZINE Nasal Solution, in 1-oz. dropper bottles, 0.1%. TYZINE Nasal Spray, in plastic bottles, 15 cc., 0.1%. TYZINE Pediatric Nasal Drops, in 1/2-oz. bottles, 0.05%, with calibrated dropper for precise dosage.

1. Graves, J. W.: Eye, Ear, Nose & Throat Month. 34:670, 1955. 2. Menger, H. C.: New York J. Med. 55:812, 1955. 3. Neistadt, I.: A.M.A. Arch. Otolaryng. 62:143, 1955.

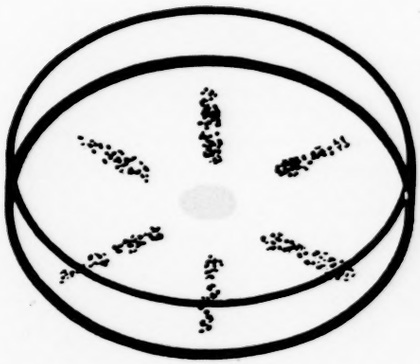
Pfizer

PFIZER LABORATORIES

Division, Chas. Pfizer & Co., Inc.

Brooklyn 6, New York

IN URINARY TRACT INFECTIONS



sensitivity studies suggest

FURADANTIN's "broad spectrum of antibacterial and antiprotozoal activity *in vitro*"[1] makes it important to include this agent, routinely, in sensitivity tests. Organisms commonly resistant to most of the antibiotics, notably Proteus and Pseudomonas, are often inhibited by FURADANTIN.[2]

Furadantin®

BRAND OF NITROFURANTOIN

". . . may be unique as a wide-spectrum antimicrobial agent that is bactericidal, relatively nontoxic, and does not invoke resistant mutants."[3]

clinical cures confirm

"Clinical studies have demonstrated rapid clinical response of cases of cystitis, prostatitis, and pyelonephritis."[4]

Typical Timetable of Furadantin Effectiveness

In *30 minutes,* antibacterial concentrations in urine.

In *24 hours,* turbid urine is usually clear.

In *3 to 5 days,* most patients are symptom-free and the urine is free of pus cells.

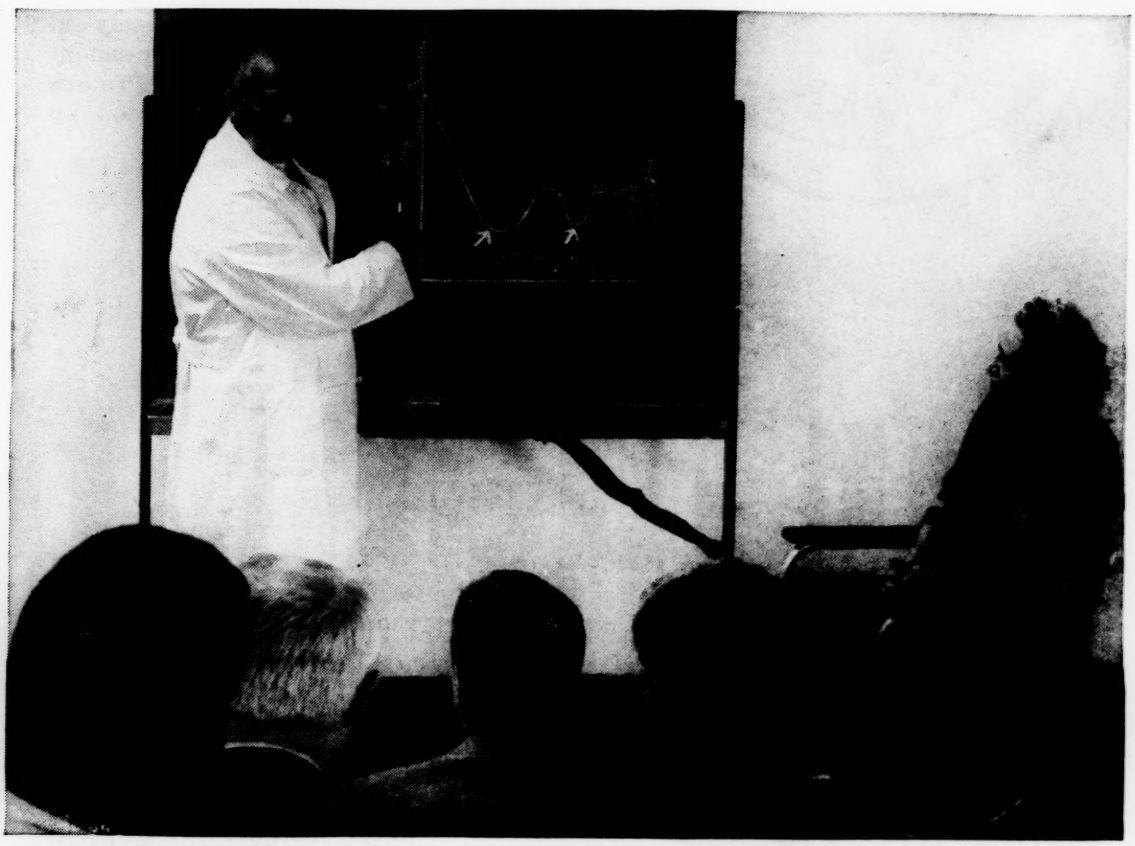
In *one week,* the urine is usually sterile.

SUPPLIED: Tablets, 50 and 100 mg., bottles of 25 and 100.
Oral Suspension, 5 mg. per cc., bottle of 118 cc.

REFERENCES: 1. Mintzer, S., et al.: Antibiotics 3:151, 1953. 2. Felton, F. C., and Kemp, A. P.: J. Urol. 73:718, 1955. 3. Walsbren, B. A., and Crowley, W.: A.M.A. Arch. Int. M. 95:653, 1955. 4. Flippin, H. F., and Eisenberg, G. M.: Antimicrobial Therapy in Medical Practice, Philadelphia, F. A. Davis Company, 1955, p. 40.

EATON LABORATORIES, Norwich, N.Y. NITROFURANS a new class of antimicrobials neither antibiotics nor sulfas

adjusts anticoagulant-depressed prothrombin time



MAJOR ADVANTAGES: Action detectable within 15 minutes, prothrombin time normalized within 4 to 12 hours, bleeding checked in 3 to 6 hours.

EMULSION OF Mephyton®

(VITAMIN K_1 INJECTION, MERCK)

"It is shown that oil-soluble vitamin K_1 is more effective than any other agent now available in combating drug-induced hypoprothrombinemia."[1] However, the usefulness of Mephyton lies not only in overcoming bleeding emergencies, but also in adjusting upwards to safe therapeutic levels unduly prolonged prothrombin times. Mephyton permits the clinician to do this easily and without gross changes in the regular anticoagulant dosage. . . . "no untoward effects have been observed to follow the administration of vitamin K_1."[1]

INDICATIONS: Hypoprothrombinemia due to Dicumarol®, Cumopyran®, Tromexan®, Hedulin®, 'Dipaxin', and other prothrombin depressing agents; also when due to antibiotics, salicylates, obstructive jaundice, hepatic disease, and impaired gastrointestinal absorption.

SUPPLIED: In boxes of six 1-cc. ampuls, 50 mg. of vitamin K_1 per cc.

1. Gamble, J. R., *et al.*, *Arch. Int. Med.* 95:52, January 1955.

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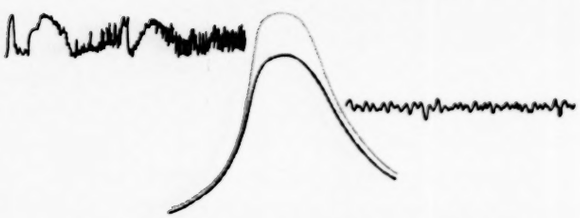

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1. Doyle, P. J., and Livingston, S.: J. Pediat. *43*:413 (Oct.) 1953.
2. Livingston, S., and Petersen, D.: To be published.
3. Pence, L. M.: Texas State J. Med. *50*:290 (May) 1954.

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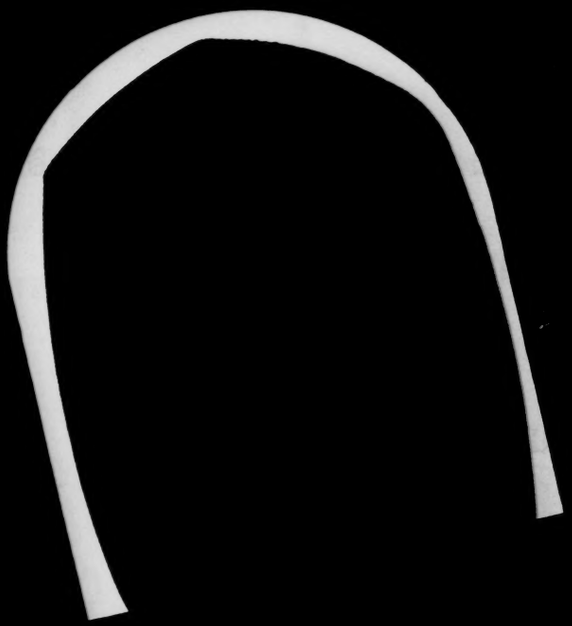

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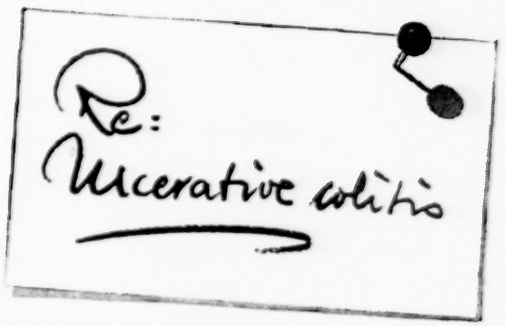

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1. BARGEN, J. A.: "Present Status of Hormonal and Drug Therapy of Ulcerative Colitis", South. M. J. *48*: 192 (Feb.) 1955.
2. BARGEN, J. A. and KENNEDY, R. L. J.: "Chronic Ulcerative Colitis in Children", Postgrad. Med. *17*: 127 (Feb.) 1955.
3. MORRISON, L. M.: "Response of Ulcerative Colitis to Therapy with Salicylazosulfapyridine", J. A. M. A. *151*: 366 (Jan. 31) 1953.

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1. Bollet, A. J., Black, R., and Bunim, J. J.: *J.A.M.A.* **158**: 459, June 11, 1955.

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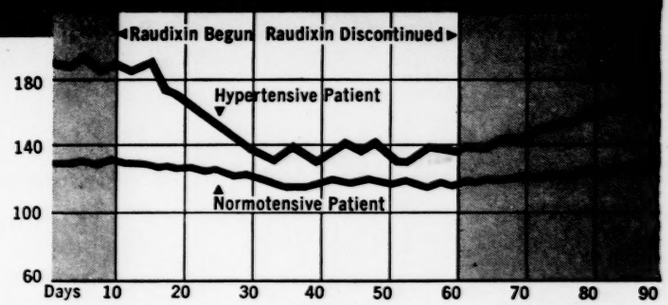

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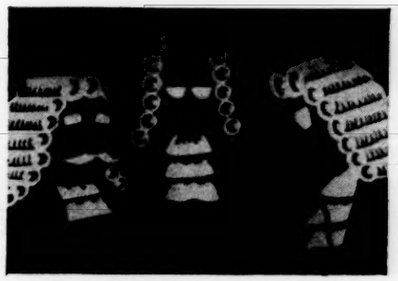

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1. Busse, E.A.: Treatment of Rheumatoid Arthritis by a Combination of Cortisone and Salicylates. Clinical Med. 11:1105 (Nov., 1955).
2. Roskam, J., VanCawenberge, H.: Abst. in J.A.M.A., 151:248 (1953).
3. Coventry, M.D.: Proc. Staff Meet., Mayo Clinic, 29:60 (1954).
4. Holt, K.S., et al.: Lancet, 2:1144 (1954).
5. Spies, T.D., et al.: J.A.M.A., 159:645 (Oct. 15, 1955).

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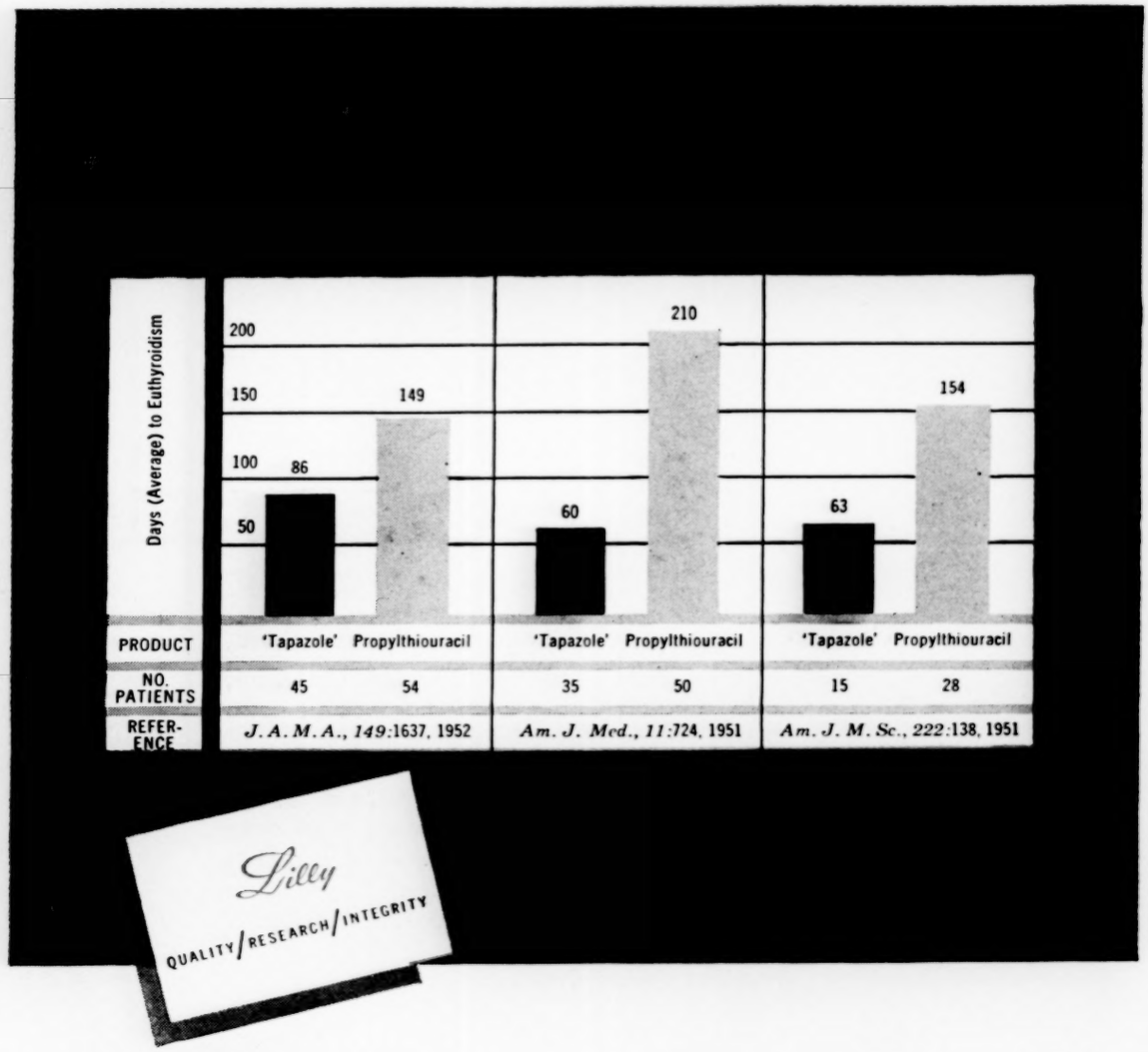

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The American Journal of Medicine

VOL. XX APRIL, 1956 NO. 4

Editorial

Serologic Reactions in Rheumatoid Arthritis

DURING the past decade an increasing effort has been made to elucidate the etiology and pathogenesis of the various types of arthritis. To implement this effort there have been established the Arthritis and Rheumatism Foundation, the National Institute for Arthritis and Metabolic Diseases of the United States Public Health Service and an increasing number of groups interested in rheumatism at the various teaching centers.

Recent research efforts have resulted in important advances in our understanding of the fundamental nature of the connective tissues and their reactions in inflammation and repair. The administration of adrenocortical hormones to patients with rheumatoid arthritis enables us to suppress the host response and results in prompt disappearance of all manifestations of the disease except irreversible structural changes and certain serologic reactions. It is possible that these serologic reactions are directly related to the pathogenic process and their study may represent a direct approach to the problem of etiology and pathogenesis. Considerable confusion still exists concerning the mechanism and meaning of these serologic reactions. It is the purpose of this editorial to outline what is known and to define unresolved problems and certain prospective goals.

The evaluation of these tests for diagnosis has been limited because of the difficulty of clinical diagnosis in patients with early, mild or atypical rheumatoid arthritis. Nevertheless, regardless of the technic used, a large majority of patients with typical disease, and particularly those with rheumatoid nodules (97 per cent), give positive serologic reactions.[1-4]

Three serologic test systems have been investigated. In the streptococcus agglutination test (S.A.), several strains of group A hemolytic streptococci are incubated with serial dilutions of the patient's serum in saline solution. Macroscopic clumping of the organisms constitutes a positive reaction.[1-3] In the sensitized sheep cell test (S.S.C.), sheep red blood cells, to which have been added non-agglutinating amounts of rabbit sheep cell antiserum, are incubated with serial dilutions of the patient's serum in saline solution.[16] The reaction is positive when the quantity of the patient's serum necessary to agglutinate these sensitized sheep cells is less than sixteen times that necessary to agglutinate untreated sheep cells. The need for the comparison between these two dilutions is eliminated if naturally occurring heterophil antibodies in the patient's serum are previously absorbed.[5] When certain animal serums are used as a diluent rather than saline solution, the titer of a positive serum is raised from fourfold to five hundred fold. In this situation the criterion for a positive reaction is at least a fourfold potentiation for the aliquot diluted in animal serum as compared with that diluted in saline solution.[6]

In the third and most recent test, the so-called FII system, sheep cells treated with tannic acid are coated with a solution of pooled human gamma globulin (Cohn fraction II, or FII) and then added to serial dilutions of the patient's serum in saline solution. A positive reaction occurs when there is maximal agglutination of the treated sheep cells by a dilution of at least 1:56 of the patient's serum.[7]

Several modifications of the S.S.C. test have recently been employed using crude fractions of the serum to be tested. The water-insoluble fraction (euglobulin) has been reported to con-

tain the agglutinating factor in rheumatoid arthritics.[9] It has been reported that the euglobulin fraction of rheumatoid serum may give a positive reaction when the whole serum gives a negative reaction.[8] The water-soluble fraction, that soluble in low salt concentrations (pseudoglobulin), has been reported to contain the agglutinating factor when positive reactions are given by the serums of patients who do not have rheumatoid arthritis.[9] This latter finding may enable one to distinguish a false positive reaction.

The reaction of serum with each of the systems mentioned has not yet been shown to have the characteristics of an antigen-antibody reaction. Therefore, the factor in rheumatoid serum will be called the reactor rather than antibody, the substance with which it reacts the reactant rather than antigen.

The Reactant. If the assumption is granted that the sheep red blood cell and the hemolytic streptococcus serve as inert carriers in these reactions, the common feature of the three systems is that the carrier is first coated with a constituent of serum. In the FII system the factor responsible for the activity of pooled human gamma globulin has been found in the gamma globulin of only certain human serums.[7] In the case of the streptococcus, this is in the water-soluble fraction (pseudoglobulin) found in normal adults.[10] In the case of the S.S.C. system, it is in the rabbit antisheep cell serum (amboceptor).

In support of this assumption of the inert role of these carriers is the observation that an egg albumin-antiegg albumin[11] and a brucella-antibrucella system[12] can react with the factor from serum giving a positive reaction. Cohn fraction II of certain animal or human serums, consisting mainly of gamma globulin, has been found to reduce the titer of serum giving a positive reaction in all of these tests.[13,14] It is possible to explain this action of gamma globulin as competition for circulating rheumatoid factor (reactor) between the gamma globulin in solution and that serum constituent coated on the carrier.

It appears, then, that the reactant is contained in the gamma globulin fraction of some human serums and in the antiserums to a seemingly unrelated group of antigens. It should be possible, in time, to characterize this material.

The Reactor or Serum Rheumatoid Factor. Fraction III (Heller) of serum giving positive serologic reactions contains the agglutinating factor for the S.S.C. and FII systems.[13] This fraction (Heller fraction III) is predominately beta globulin but contains small amounts of alpha and gamma globulins as well as some albumin. The titer obtained in any test system seems to bear no direct relationship to the absolute amounts of any of these proteins. The chemical nature of the factor has not been established. However, it has been shown that the protein nitrogen may be reduced to 70 per cent, phospholipids to 38 per cent and total cholesterol to 1 per cent of their original Heller fraction III values without loss of titer.[13] Separation by paper electrophoresis places the reactor in the gamma globulins.[10] The relation of reactor to complement is not fully established. However, inactivation of the components of complement has failed to reduce the S.S.C. titer of rheumatoid arthritis serums.[15]

The streptococcus agglutination reaction has been found to depend on two serum factors. The first is contained in the water-soluble fraction (pseudoglobulin) of adult serum and is found in the serums of both normal and rheumatoid subjects. The second and specific factor is contained in the water-insoluble fraction (euglobulin) of serum of patients with rheumatoid arthritis.[10] Further identification of these two factors has not been made but the possibility that the pseudoglobulin is a source of reactant gamma globulin is under investigation.

According to one group, the euglobulin fraction of all normal human serum contains inhibiting activity to the S.S.C. system. This is said to be lacking in the euglobulin fraction of serum from persons with rheumatoid arthritis. A test based on the inability of euglobulin obtained from the serum of patients with rheumatoid arthritis to inhibit a system giving positive reactions has been suggested.[8] Ethanol fractionation of serums giving a positive reaction has failed, however, to raise the titer obtained from Heller fraction III compared to the whole serum. Similar fractionation of serums giving a negative reaction from patients with rheumatoid arthritis has failed to produce a subfraction giving a positive reaction.[13]

Occasionally a serum or its Heller fraction III may give a positive reaction with one and not the other system, but usually it gives a reaction with all three.[13] Differential absorption studies have shown that the FII cell will absorb all the S.A. factor and all the S.S.C. factor from the

serum of a patient with rheumatoid arthritis.[13,14] Absorption of a serum with sensitized sheep cells will only incompletely remove the factor for the FII coated cells.[13] The S.S.C. factor of serum is not affected by prior absorption of serum with streptococci.[10] Complete absorption of the S.S.C. factor from the water-insoluble fraction (euglobulin) of a serum giving a positive serologic reaction caused a fall but not a complete disappearance of the S.A. titer.[10] These differential absorption studies suggest that there may be more than one reactor material present or that only a portion of a single reactor acts with some test systems.

In summary, the reactor can be partially separated from whole serum by cold ethanol fractionation, by water fractionation and by paper electrophoresis. A beginning of chemical characterization of the reactor has been made. The easily standardized FII system and the technic of inhibition reactions should stimulate further work on the characteristics of the reactor-reactant mechanism.

In the foreseeable future we may be able to determine the character of the fraction of human gamma globulin which reacts with the material found circulating in the blood of most patients with rheumatoid arthritis. It seems, therefore, that the hypothesis that rheumatoid arthritis is a product of hypersensitivity involving a component of host tissue may be found to have further supporting evidence.

WALLACE EPSTEIN, M.D. *and*
CHARLES RAGAN, M.D.
Department of Medicine,
Columbia University College of Physicians and Surgeons,
New York, New York

REFERENCES

1. NICHOLLS, E. E. and STAINSBY, W. J. Streptococcal agglutinins in infectious arthritis. *J. Clin. Investigation*, 10: 323, 1931.
2. MCEWEN, C., BUNIM, J. J. and ALEXANDER, R. C. Bacteriologic and immunologic studies in arthritis. II. Results of various immunologic tests in different forms of arthritis. *J. Lab. & Clin. Med.*, 21: 465, 1936.
3. DAWSON, M. H., OLMSTEAD, M. and BOOTS, R. H. Agglutination reactions in rheumatoid arthritis. I. Agglutination reactions with streptococcus hemolyticus. *J. Immunol.*, 23: 187, 1932.
4. BUNIM, J. J., SOKOLOFF, L., WILLIAMS, R. R. and BLACK, R. L. Rheumatoid arthritis: a review of recent advances in our knowledge concerning pathology, diagnosis and treatment. *J. Chronic Dis.*, 1: 168, 1955.
5. HELLER, G., JACOBSON, A. S. and KOLODNY, M. H. A modification of the hemagglutination test for rheumatoid arthritis. *Proc. Soc. Exper. Biol. & Med.*, 72: 316, 1949.
6. HELLER, G., JACOBSON, A. S., KOLODNY, M. H. and SCHUMAN, R. L. The hemagglutination test for rheumatoid arthritis. I. An immunological analysis of the factors involved in the reaction. *J. Immunol.*, 69: 27, 1952.
7. HELLER, G., JACOBSON, A. S., KOLODNY, M. H. and KAMMERER, W. H. The hemagglutination test for rheumatoid arthritis. II. The influence of human plasma fraction II (gamma globulin) on the reaction. *J. Immunol.*, 72: 66, 1954.
8. ZIFF, M., BROWN, P., BADIN, J. and MCEWEN, C. A hemagglutination test for rheumatoid arthritis with enhanced sensitivity using the euglobulin fraction. *Bull. Rheumat. Dis.*, 5: 75, 1954.
9. SVARTZ, N. and SCHLOSSMAN, K. A serum cold precipitable hemagglutinating factor in rheumatoid arthritis. *Acta med. Scandinav.*, 149: 83, 1954.
10. LAMONT-HAVERS, R. W. Nature of serum factors causing agglutination of sensitized sheep cells and group A hemolytic streptococci. *Proc. Soc. Exper. Biol. & Med.*, 88: 35, 1955.
11. VAUGHAN, J. Behavior of the agglutination activating factor of rheumatoid arthritis with immune precipitates. Proc. Annual Meet., American Rheumatism Association, Atlantic City, N. J. June, 1955.
12. FOZ, A., BATALLA, E. and ESPACIO, L. Auto-antibodies in rheumatoid arthritis. A simple method of demonstration with possible diagnostic application. *Ann. Rheumat. Dis.*, 14: 313, 1955.
13. HELLER, G., KOLODNY, M. H., LEPOW, I. H., JACOBSON, A. S., RIVERA, M. E. and MARKS, G. H. The hemagglutination test for rheumatoid arthritis. IV. Characterization of the rheumatoid agglutinating factors by analysis of serum fractions prepared by ethanol fractionation. *J. Immunol.*, 74: 340, 1955.
14. EPSTEIN, W. and RAGAN, C. Unpublished data.
15. LAMONT-HAVERS, R. W. Studies on the sensitized sheep cell agglutination reaction of rheumatoid sera. Presented in part requirement for the Diploma Course in Medicine, McGill University, Montreal, Canada, 1952.
16. ROSE, H. M., RAGAN, C., PEARCE, E. and LIPMAN, M. O. Differential agglutination of normal and sensitized sheep erythrocytes to sera of patients with rheumatoid arthritis. *Proc. Soc. Exper. Biol. & Med.*, 68: 1, 1948

Clinical Studies

The Hemagglutination Test for Rheumatoid Arthritis*

III. Clinical Evaluation of the Sheep Erythrocyte Agglutination (S.E.A.) Test and the Gamma Globulin (FII) Tests

ABRAHAM S. JACOBSON, M.D., WILLIAM H. KAMMERER, M.D., JULIUS WOLF, M.D., WALLACE V. EPSTEIN, M.D. *and* GEORGE HELLER,† PH.D.

New York, New York

In the absence of precise clinical criteria for the diagnosis of rheumatoid arthritis and of knowledge of its etiology, the continuing interest in a serologic test for this disease is understandable. Several hemagglutination tests have recently been described which possess varying degrees of specificity and sensitivity.[1–9] Waaler[1] in 1940 found that the serums of some patients with rheumatoid arthritis enhanced the agglutination of sheep red cells by rabbit antisheep cell serum. Rose et al.[2] described a test based on the observation that sheep erythrocytes sensitized with non-agglutinating quantities of rabbit antisheep erythrocyte serum were agglutinated by higher dilutions of rheumatoid serums than were unsensitized cells. This test was considered positive when the difference in the agglutination of sensitized erythrocytes over unsensitized ones was sixteenfold. A modification consisting of previous absorption of the naturally occurring sheep cell antibodies in human serum did not significantly reduce the titers obtained with sensitized sheep cells when rheumatoid serums were tested. This increased the sensitivity of the test without decreasing specificity[3,12] and made a differential titer unnecessary.

In 1953 the first of the two tests analyzed in this report was developed.[4] It was found that if sheep serum was used as a diluent for serum from patients with rheumatoid arthritis, the agglutination titers were four to 500 times greater than those obtained when saline solution was used as the diluent. In the course of these latter studies it was observed that certain human serums used as a diluent for the serum under study had the effect of reducing the final titer. Pooled gamma globulin from patients with rheumatoid arthritis or normal subjects was found to have this same capacity. As a result of these observations, the second test subjected to analysis in the present study was developed. In the latter test pooled gamma globulin is bound to sheep red cells by means of prior tannic acid treatment of the cells. This reagent is then added to serial dilutions of the patient's serum in saline. Analysis of our experience in the use of these two serologic tests in 1,576 patients is presented in this paper.

CLINICAL MATERIAL

The case material of this study is composed of patients at the Bronx Veterans Administration Hospital and the private patients of one of the authors (W. H. K.). During the early part of the study the former were observed by two of the authors (A. S. J. and W. H. K.) and more recently by another two (J. W. and W. V. E.). Thus the study consists of three groups of patients observed by different individuals. The results in each group were so similar that they were combined for the purposes of this paper. Serums received in the laboratory were assigned code numbers, and no indication of the working diagnosis was available to those reading the tests. Each patient was seen by one of us and a diagnosis assigned in the absence of knowledge of the serologic results. The criteria of the American Rheumatism Association[10] and the classi-

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† Deceased.

fication developed by Steinbrocker, Traeger and Batterman[11] were used.

The diagnosis of rheumatoid arthritis in its early stages is difficult. It was our practice to consider most of the cases of mild, recurring peripheral polyarthritis with true synovitis as rheumatoid unless there were specific indications for another diagnosis. In the unclassified group we placed the cases of unexplained monarticular arthritis and patients with one attack of mild arthritis or arthralgia of atypical nature. The diagnosis of rheumatoid arthritis and rheumatoid spondylitis in the same patient was limited to those whose rheumatoid arthritis had its clinical inception after puberty and who showed definite evidence of destructive lesions involving sacroiliac and/or spinal apophyseal joints as well as peripheral joints distal to elbows and knees. Patients with associated psoriasis and arthritis were classified into groups comprising those showing distinct distal interphalangeal joint involvement and those whose destructive arthritis did not extend beyond the proximal interphalageal joints. The presence of minor degrees of osteoarthritic changes has been ignored in the patients with rheumatoid arthritis, rheumatoid spondylitis or gout; the latter diagnoses were considered primary.

The predominance of males in our cases is a natural consequence of the Veterans Administration Hospital patient population.

Technic of the Tests

Preparation of all reagents and details of the test procedures have been previously published.[13] A brief outline of the two tests follows:

Sheep Erythrocyte Agglutination (S.E.A.) Test Procedure. Sheep erythrocyte samples are selected which exhibit a 1/2,000 basic agglutination titer with a stock preparation of antisheep erythrocyte serum. The erythrocyte (R.B.C.) suspensions are sensitized with 1/20,000 dilution of the antiserum ($\frac{1}{10}$ of basic agglutination titer) for use in the diagnostic test. Two per cent sheep serum (complement inactivated) is chosen on condition that: (1) it is compatible with the unsensitized R.B.C.; (2) its normal conglutinin property is sufficiently low so as not to agglutinate sensitized R.B.C. reagent alone; (3) it possesses the property of specifically enhancing the agglutination titer of a known positive-reacting rheumatoid (R) reference serum to a maximal degree. Naturally occurring sheep erythrocyte agglutinins are eliminated from complement inactivated test serums by absorption. Twofold serial dilutions of test serum aliquots are concurrently prepared in 0.5 ml. volumes of saline and in 0.5 ml. volumes of 2 per cent sheep serum in a dilution range of 1/7 through 1/56,000. Equal volumes of the sensitized sheep erythrocyte suspension are added to all dilutions including appropriate control tubes. The test is considered positive if the titer obtained in the sheep serum diluent is fourfold or greater than that of the aliquot diluted in saline solution.

The Gamma Globulin or Fraction II (FII) *Test Procedure.* Fraction II solution is prepared in 0.15 molar phosphate buffered saline solution, pH 8.0, from the lyophilized powder* derived from pooled human plasma. Sheep R.B.C. in buffered saline are added dropwise to a 1/40,000 dilution of tannic acid, heated, centrifuged and then reconstituted in buffered saline. The tannic acid–treated R.B.C. suspension is sensitized with a fraction II solution in a concentration equivalent to twice that necessary to produce a maximum titer of a standard R serum. Then 0.5 ml. portions of the FII sensitized cell suspension are added to equal volumes of the test serum diluted in buffered saline solution in a dilution range 1/28 through 1/56,000. Appropriate controls are used. After overnight refrigeration the tests are read by placing the tube support on a horizontally placed X-ray viewing box. Agglutination is observed by viewing the reaction from above. A positive reaction is one in which maximal agglutination is observed in the 1/28 or greater dilutions. The ease of reading this test and the sharpness of the end point are illustrated in Figure 1. The red cells in dilutions of a control serum from non-rheumatoid patients (N serum) are seen as tight buttons as compared to a uniform blanket of cells in the rows containing serial dilutions of the R serums.

RESULTS

Table I is an analysis of all cases included in this study. The results of the first tests performed in each patient were used for the tabulation. It should be noted that not all serums tested by the S.E.A. procedure were tested by the FII procedure and that the first FII test was not carried out in all cases at the same time as the first S.E.A. test.

For purposes of comparison we have divided all cases into four groups:

1. Peripheral rheumatoid arthritis without regard to duration, stage, class or "degree" of activity. This group is subject to recognized inaccuracies of diagnosis. We found that 62 per cent of 331 patients had a positive reaction to the S.E.A. test, whereas 69 per cent of 180 patients had a positive reaction to the FII test. Of forty-six serums of patients with rheumatoid arthritis tested simultaneously with both tests, thirty-two gave positive reactions to both, five gave positive reactions to the S.E.A. test and negative reactions to the FII, two gave positive reactions to

* The lyophilized powder, obtained through the generosity of the American Red Cross, was prepared in the Human Blood Products Dept. of E. R. Squibb & Sons, New Brunswick, N. J.

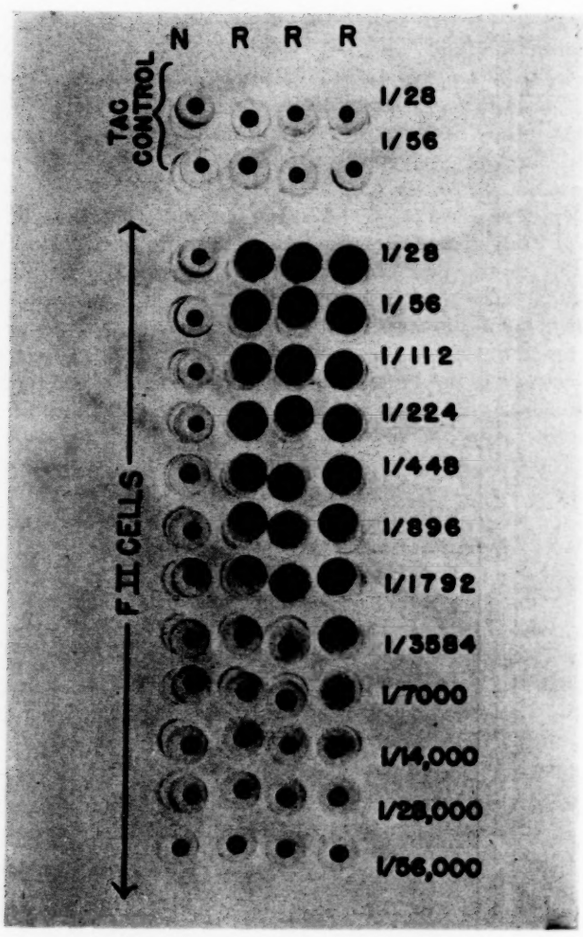

FIG. 1. Appearance of FII (gamma globulin) sensitized sheep erythrocytes in dilutions of serums from three patients with rheumatoid arthritis and a non-rheumatoid control subject.

the FII test and negative reactions to the S.E.A., and seven gave negative reactions to both tests.

2. The second group includes three subdivisions: rheumatoid spondylitis, psoriasis associated with arthritis, and a combination of rheumatoid arthritis and rheumatoid spondylitis. Rheumatoid spondylitis was notable for its low degree of positivity with only 2.1 per cent of ninety-five patients having positive reactions to S.E.A. tests, and only one of twenty-eight of these same patients having positive reactions to FII tests. The group with both spondylitis and peripheral rheumatoid arthritis occupies an intermediate position with 26 per cent positive reaction to the S.E.A. test and 33 per cent positive reaction to the FII. Psoriasis associated with arthritis with or without distal interphalangeal joint involvement showed about the same incidence of positivity with combined values of 22 per cent S.E.A. and 29 per cent positive FII tests. The values for these three clinical entities combined are 10 per cent positive reaction to the S.E.A. test and 15 per cent positive reaction to the FII test.

3. Of other arthritides, no group of any significant size had an incidence of positive reactions to tests greater than 10 per cent by the S.E.A. or 9 per cent by the FII. It may be of interest that only one of ten patients with disseminated lupus erythematosus tested with the S.E.A. procedure had a positive reaction, but that two of three such cases tested with the FII test had a positive reaction. One patient with diffuse arthralgia secondary to apresoline® therapy for hypertension had a positive reaction to the S.E.A. test but not to the FII test. None of the sixty-two patients with gout had positive reactions to the S.E.A. test and only one of thirty-four patients tested had a positive reaction to the FII test. Of our cases of scleroderma, dermatomyositis and polyarteritis nodosa, only one patient with polyarteritis had a positive reaction to the S.E.A. test. The one patient with osteoarthritis with positive reactions to both tests, as well as the one patient with allergic arthritis with a positive reaction to the FII test alone, had positive reactions to serologic tests for syphilis at the time these tests were done. Both of these patients when subsequently tested had negative reactions to the S.E.A. and FII tests. In our only case of rheumatic fever in which these were positive reactions to both tests, there were no follow-up studies; however, in the one case with a positive reaction to the FII and not the S.E.A. there was a subsequent negative reaction to the FII.

In the group of 119 cases designated as unclassified joint diseases, 10 per cent of 119 patients tested had positive reactions to the S.E.A. test, whereas 9 per cent had positive reactions to the FII test. Only long-term follow-up will decide whether those positive tests represent rheumatoid arthritis or false-positive reactions. Of forty-eight cases of unclassified joint disease studied simultaneously with both tests, forty-four patients had negative reactions to both tests whereas four had positive reactions to the FII test and negative reactions to the S.E.A. test.

4. The patients included in the final group exhibited no arthritic complaints and included hospitalized patients as well as those seen in the Outpatient Service. Fifteen of 560 patients (3 per cent) had positive reactions to S.E.A. tests whereas eight of 376 (2 per cent) of these indi-

TABLE I

ANALYSIS OF RESULTS IN ALL CASES

Disease	Total No. of Cases	No. S.E.A.-Positive on First Test	Per cent S.E.A.-Positive on First Test	No. of FII Tests	No. FII-Positive on First Test	Per cent FII-Positive on First Test
1. Rheumatoid arthritis	331	205	62	180	124	69
2. Rheumatoid arthritis and rheumatoid spondylitis	27	7	26	6	2	33
Rheumatoid spondylitis	95	2	2.1	28	1	3.6
Psoriasis associated with arthritis:						
With terminal interphalangeal joint involvement	12	3	25	7	2	28
Without terminal interphalangeal joint involvement	15	3	20	6	2	33
3. Other Arthritides:						
Osteoarthritis	103	5	4.8	53	3	5.7
Gout	62	0	0	34	1	2.9
Rheumatic polyarthritis	72	1	1.4	33	2	8.7
Palindromic rheumatism	6	0	0	4	0	0
Reiter's syndrome	12	0	0	8	0	0
Disseminated lupus erythematosus	10	1	10	3	2	66
Traumatic arthritis	27	1	3.7	6	0	0
Allergic arthritis	17	0	0	9	1	11
Infectious arthritis	24	0	0	13	1	8
Neuropathic arthritis	3	0	0	3	0	0
Apresoline arthritis	1	1	100	1	0	0
Pulmonary osteoarthropathy	7	0	0	1	0	0
Shoulder-hand syndrome	5	0	0	5	0	0
Myositis	2	0	0	1	0	0
Arthritis secondary to sarcoidosis	2	0	0	2	0	0
Intermittent hydrarthrosis	3	0	0	...	...	....
Scleroderma	6	0	0	...	...	....
Polyarteritis nodosa	5	1	20	...	...	....
Psychogenic arthritis	27	0	0	10	0	0
Dermatomyositis	1	0	0	...	...	....
Arthritis with ulcerative colitis	7	0	0	...	...	....
Dupuytren's contracture	1	0	0	1	0	0
Tumor of bone	1	0	0	1	0	0
Psoriasis, no arthritis	13	1	8	12	0	0
Unclassified joint diseases	119	12	10	54	5	9
4. No arthritic complaints	560	15	3	376	8	2

viduals tested with the FII procedure had positive reactions. Of fifty-three patients tested simultaneously with both procedures, forty-nine had negative reactions to both tests, one had a positive reaction to both tests (a case of healed subacute bacterial endocarditis) and three had positive reactions to the FII test and negative reactions to the S.E.A. These last three include one case of Hodgkin's disease, one of arteriosclerotic heart disease and one instance of hyperglobulinemia (7.0 Gm. per cent) secondary to multiple chronic infections.

Rheumatoid Arthritis According to Stage and Class. The categories used in Tables II and III are based on criteria for severity of rheumatoid arthritis by stages, and functional incapacitation by classes progressing from least to greatest (I to IV).[11] These figures again represent only those determinations carried out the first time the patient was seen. In most instances the FII test was obtained at the same time as the S.E.A.

It is undoubtedly true that the certainty of clinical diagnosis increased as the stage and class of rheumatoid arthritis progressed. Therefore the

rising incidence of positive tests by both criteria may bear a relation to the homogeneity of the subgroup as well as to a property of the disease.

Rheumatoid Arthritis According to Sex. Table IV presents the incidence of positive reactions to first and multiple S.E.A. tests according to sex.

TABLE II
VARIATION OF TESTS WITH STAGE OF RHEUMATOID ARTHRITIS

Stage at First Test	No. of Cases	No. S.E.A.-Positive	Per cent S.E.A.-Positive	No. of FII Tests	No. FII-Positive	Per cent FII-Positive
I	43	28	65	23	16	69
II	29	23	79	4	4	100
III	15	14	93	15	13	87
IV	38	32	84	2	2	100
No data	11	7	64	3	0	0

TABLE III
VARIATION OF TESTS WITH FUNCTIONAL INCAPACITATION

Class at First Test	No. of Cases	No. S.E.A.-Positive	Per cent S.E.A.-Positive	No. of FII Tests	No. of FII-Positive	Per cent FII-Positive
I	8	5	63	8	3	38
II	26	22	85	23	20	87
III	16	11	69	13	11	85
IV	1	1	100	1	1	100
No data	3	2	66	3	1	33

TABLE IV
RELATION OF TEST TO SEX

Sex	No. of Cases	No. S.E.A.-Positive on First Test	Per cent S.E.A.-Positive on First Test	Per cent S.E.A.-Positive on Multiple Tests
Male.....	114	85	75	80
Female...	133	75	56	65

It will be noted that there was a higher incidence of positive titers in males as compared with females; this incidence persisted even when multiple tests were performed.

Prior Duration of the Disease. Table V shows the duration of clinical rheumatoid arthritis prior to the first test. A general increase in the incidence of positive tests is noted with increasing duration of the disease. Two patients, however, had reactions to positive tests simultaneously with the onset of clinical symptoms. These two patients first noticed joint complaints while in the Bronx Veterans Administration Hospital for other reasons. A sixty-three year old white man, admitted for a stasis dermatitis, had a tender swelling of his right wrist eighteen hours before blood was drawn for the first serologic tests.

TABLE V
RELATION OF TESTS TO DURATION OF DISEASE

Duration	No. of S.E.A. Tests	No. S.E.A.-Positive	Per cent S.E.A.-Positive	No. of FII Tests	No. FII-Positive	Per cent FII-Positive
Less than 6 mo..	36	21	58	9	6	66
6 mo. to 1 yr....	28	19	68	5	2	40
1–5 yr.....	67	66	76	10	9	90
5–10 yr.....	55	47	85	9	8	89
Over 10 yr.....	70	49	70	12	8	67
No data.....	25	17	68	3	3	100

These tests showed titers with the S.E.A. test of 1/448 in saline solution and 1/14,000 in sheep serum (thirty-two fold potentiation) and of 1/3,584 with the FII test. X-rays revealed no abnormality. The patient has continued to have exacerbations of arthritis, with development of synovitis of proximal interphalangeal joints. Reactions to both tests remain positive. There were no prior articular complaints in this case.

The second patient, a sixty-four year old white man, was admitted because of recurrent generalized psoriasis. The results of the S.E.A. test on serum drawn the same day as the patient's first complaint of aching in the left shoulder, elbow and wrist was 1/14 in saline and 1/224 in sheep serum (sixteenfold potentiation). This case has progressed to deforming arthritis of the hands, knees and elbows, with distal interphalangeal joint development. Reactions to both the S.E.A. and subsequent FII tests have remained positive.

Rheumatoid Nodules. Table VI is a study of patients diagnosed as having rheumatoid arthritis in whom subcutaneous nodules were present at the time of their first test. In some instances these nodules were biopsied and confirmed microscopically.

The incidence of positive reactions to first tests here is strikingly different from the prior analysis by stage alone. In general we have found it distinctly unusual to obtain a negative reaction to testing in a patient with rheumatoid arthritis with nodule formation, no matter what the apparent stage of disease progression. But again,

the high incidence of positive tests may be a matter of certainty of diagnosis due to the presence of the nodules.

Activity of Disease. Table VII presents those determinations frequently carried out in patients with rheumatoid arthritis as part of an assessment of what has been termed the "activity" of the disease. Active joint inflammation refers to heat, redness, synovial swelling, joint effusion or any combination of these objective findings. We have no explanation for the low incidence of hyperglobulinemia in this group in comparison with other studies. When the results in Table VII are compared with those in Table I, it would not seem that any of these determinations lead to groupings of significantly higher positivity.

TABLE VI
RELATION OF TESTS TO PRESENCE OF RHEUMATOID NODULES

Rheumatoid Nodules	No. of S.E.A.-Tests	No. S.E.A.-Positive	Per cent S.E.A.-Positive	No. of FII Tests	No. FII-Positive	Per cent FII-Positive
Present	84	80	95	13	12	92
Absent	204	116	56	21	15	71
No data	47	27	57	..	..	..

Response to Therapy. It was naturally of interest to study possible changes in these tests with response to therapy. Grade responses are according to the criteria accepted by The American Rheumatism Association.[11] Table VIII records changes with therapy in those patients with rheumatoid arthritis who received over 500 mg. of gold, or over six months of ACTH or cortisone, or six months of physiotherapy (usually combined with salicylates). Unfortunately the total series is small. There were insignificant changes in titer and few instances of conversion of positive to negative (or vice versa) with any grade of response. The response to these modes of therapy has not been analyzed separately because of the similarity of results.

Constancy of Positive or Negative Reactions to Tests. Table IX pursues this question further by analyzing the constancy of either positive or negative tests from the time the first test was performed in those patients who had more than three serial tests. It is apparent that the majority of patients with rheumatoid arthritis maintained their initial positive or negative reactions to the S.E.A. or FII test during the two-year period although the figures indicate that reaction to the S.E.A. test has a greater tendency to change with time than does reaction to the FII test. In the first six months after the first S.E.A. test there is an approximately equal chance of an originally positive reaction becoming negative as for an originally negative reaction becoming positive.

TABLE VII
RELATION OF TEST TO OTHER CRITERIA OF ACTIVITY

At Time of First Test	No. of Cases	S.E.A.		FII		
		No. Positive	Per cent Positive	No. of Cases	No. Positive	Per cent Positive
Active joint inflammation	32	22	69	26	20	77
Erythrocyte sedimentation rate over 15	43	33	77	37	30	81
Serum globulin 2–4 Gm. %	24	17	71	20	14	70
Serum globulin 4–6 Gm. %	3	2	67	3	2	67

TABLE VIII
RELATION OF TEST TO RESPONSE TO THERAPY

Effect on Tests	Grade Response			
	I	II	III	IV
Remained negative	4	23	3	3
Remained positive	0	13	11	5
Negative became positive	1	4	3	3
Positive became negative	3	5	0	2
Positive titer rose*	0	14	12	4
Positive titer fell*	1	16	12	3

* Change in titer refers to greater than fourfold difference for S.E.A. or one-tube difference for FII.

Quantitative Change in Titer. Figure 2 correlates titer changes with time by plotting the number of fold dilutions either greater or less than the original S.E.A. titer. It can be seen that over a two-year period 113 of 169 (67 per cent) such serial tests varied from fourfold greater to fourfold lesser dilutions. Figure 3 is the same type of analysis for a one-year period from the first FII test. This results in an almost identical scatter (sixty-eight of one hundred cases, 68 per cent) as the S.E.A. test.

Correlation of Figures 2 and 3 and Table IX would imply that for one to two years after the first of either of these tests, subsequent tests will

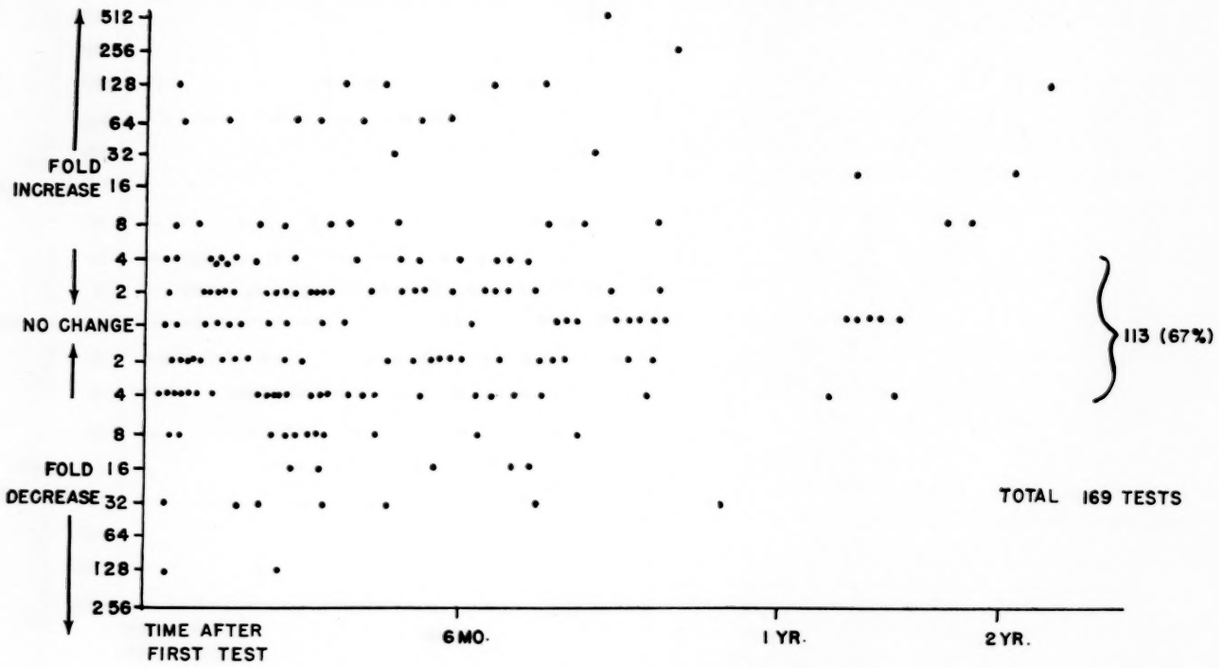

FIG. 2. Change in S.E.A. titer over original test during observation.

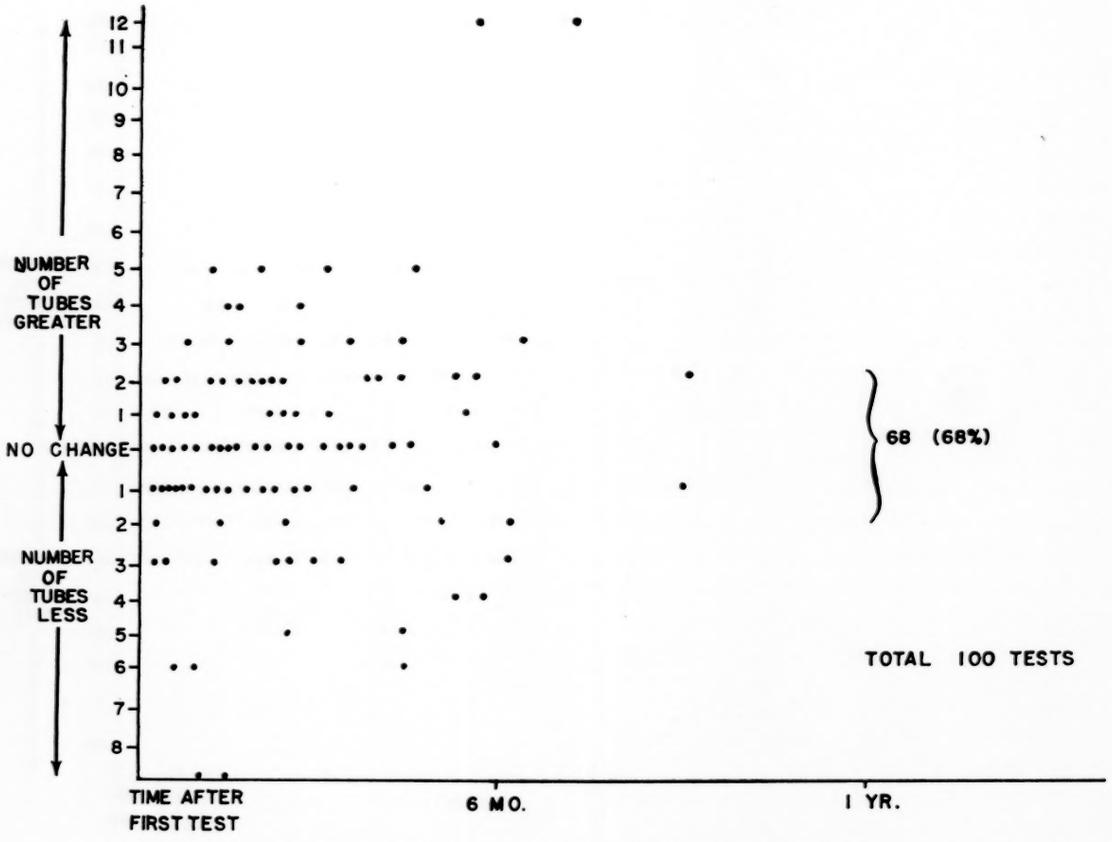

FIG. 3. Change in FII titer over original test during observation.

vary by only two dilution tubes for either test. This variation shows a greater incidence of change from positive reaction initially to negative (or vice versa) for the S.E.A. test than for the FII test.

Joint Fluid in Rheumatoid Arthritis. Table X is an analysis of these two tests on joint fluid and serum obtained within the same month. It may be seen that the joint fluid usually has the same titer or a significantly higher titer on both tests than does serum tested at about the same time.

DISCUSSION

In the light of our current knowledge, the earliest reported tests involved at least two non-specific factors in the patients' serums which were involved in the agglutination of sheep erythrocytes sensitized with their homologous antibodies:[1,3,4,14] naturally occurring heterophil antibody and conglutinin, a factor which enhances the agglutination of sensitized sheep erythrocytes. With the elimination of the heterophil constituent of the test serum by absorption, the specificity increased as the percentage of false-positive reactions decreased.[3,12] A sensitizing concentration of sheep cell agglutinins was fixed at $\frac{1}{10}$ to $\frac{1}{20}$ of the basic agglutination titer. These concentrations were found to minimize the non-specific and variable conglutinin effect and thus produce the highest specificity for the test.[4]

Despite these modifications in the test, some non-rheumatoid serums diluted in saline solution produced positive reactions. In our experience fourteen non-rheumatoid serums produced significant titers in saline solution with no rise in titer when sheep serum was used as a diluent. On the other hand, some 25 per cent of the positive reactions to the S.E.A. tests (i.e., fourfold or greater between saline solution and sheep serum diluent series) in rheumatoid serums have titers in saline solution alone which would be considered negative (up to 1/14). Therefore, only by including sheep serum as a diluent was a hemagglutination test for rheumatoid arthritis arrived at with the reported degree of specificity and sensitivity.

It has previously been demonstrated that the S.E.A. and FII reagents are heterogenetically related. This was demonstrated when a positive-reacting serum was absorbed with the S.E.A. or FII cell reagent. This treatment removed the agglutinating factors for both test reagents.[8] Serums from rheumatoid individuals may exhibit higher titers for one reagent than the other, or at times react with only one of these reagents.

The rheumatoid factor resides specifically in fraction III of positive-reacting serums.[13] By free electrophoresis this fraction is predominantly beta globulin. No correlation has been shown to exist between the agglutination factor and the beta globulin concentration.[13]

At present it is entirely within the realm of

TABLE IX
CONSTANCY OF RESULTS AFTER FIRST TEST

No. of Months after First Test	No. of Patients	No. Unchanged	No. Converted to Positive	No. Converted to Negative
1, S.E.A.	37	29	3	5
FII	20	19	0	1
2, S.E.A.	20	15	1	4
FII	10	9	0	1
3, S.E.A.	18	16	2	0
FII	9	9	0	0
4, S.E.A.	22	16	4	2
FII	11	11	0	0
5, S.E.A.	18	12	1	5
FII	8	8	0	0
6, S.E.A.	17	10	4	3
FII	4	4	0	0
7, S.E.A.	15	10	2	3
FII	3	3	0	0
8, S.E.A.	9	8	1	0
FII	3	3	0	0
9, S.E.A.	10	7	3	0
FII	2	2	0	0
10, S.E.A.	7	4	3	0
FII	1	1	0	0
11, S.E.A.	5	2	1	2
FII	0	0	0	0
12, S.E.A.	4	3	1	0
FII	3	2	1	0
1-2 yr. S.E.A.	12	9	3	0
FII	4	1	3	0

TABLE X
TITER OF JOINT FLUID COMPARED TO SERUM

Test	Same as Serum	Positive When Serum Negative	Negative When Serum positive	Significantly* Higher Than Serum	Significantly* Lower Than Serum
S.E.A. of joint fluid	27	3	4	6	1
FII of joint fluid	8	0	1	2	2

* Significant difference refers to greater than fourfold difference for S.E.A. or one-tube difference for FII.

speculation why a sheep erythrocyte bearing its homologous antibody, with or without sheep serum present, should react with such specificity with this circulating rheumatoid factor; nor is a reason for similar reactivity on the part of a sheep erythrocyte coated with gamma globulin any clearer.

Much current investigation is being directed toward the nature of rheumatoid serums negative to both of these tests. This negative quality is apparently independent of the stage, class, duration, activity or prior treatment of the disease. The following possibilities exist relative to this group of patients who appear to have rheumatoid arthritis: (1) This group represents a different disease and the rheumatoid factor as we know it is not associated with the disease process. (2) This is the same disease but our test conditions do not allow a visible reaction with the rheumatoid factors present. (3) The same rheumatoid factor is present but some inhibiting substance prevents its measurement. (4) No rheumatoid factor is present.

It seems unlikely that the negative reactions observed with some rheumatoid serums are caused by the presence of interfering substances in the test serums. The reasons for this belief follow: When whole serum or its derived gamma globulin is used to dilute a positive-reacting serum, the resulting titer is markedly reduced with either the S.E.A. or Fii cell reagent.[13] This does not represent a blocking phenomenon in which the reactors are prevented from combining by the presence of a third factor. It is apparent in this case that the reduction in titer is caused by competitive binding of similar or heterogenetically related reactors which are simultaneously present in the test system both in solution and fixed to the sheep cell reagent. Serologic assays of serum fractions have also failed to indicate the presence of a serum blocking factor. No products were obtained from the fractionation of positive-reacting and non-reacting rheumatoid serums which produced agglutination titers greater than were found in the whole unfractionated serum counterparts from which they were obtained.[13] As a result, there is no direct or inferential evidence to explain why some rheumatoid serums fail to react with the reagents used in our tests.

SUMMARY

Some 5,000 serologic tests involving two heterogenetically related test systems for the diagnosis of rheumatoid arthritis are analyzed. The tests were made in 1,576 patients with the following results, all referring to the first tests performed in individual patients: (1) Sixty-two per cent of 331 patients with rheumatoid arthritis had positive reactions to the S.E.A. test, and 69 per cent of 180 of these patients had positive reactions to the Fii test. (2) Of those patients having rheumatoid spondylitis alone, rheumatoid spondylitis and rheumatoid arthritis, or psoriasis associated with arthritis, 10 per cent had positive reactions to the S.E.A. test and 15 per cent had positive reactions to the Fii test. (3) Of all other varieties of arthritis including those with unclassified joint disease, 3 per cent had positive reactions to the S.E.A. test and 5.9 per cent had positive reactions to the Fii test. (4) Of individuals with no joint complaints, 3 per cent had positive reactions to the S.E.A. test and 2 per cent had positive reactions to the Fii test.

There is an increase in the percentage of initially positive reactions to tests in rheumatoid arthritis with increase in stage of progression or class of incapacitation. However, the substance responsible for these positive reactions to tests may be present within twenty-four hours of the first joint complaints in rheumatoid arthritis.

Male patients with rheumatoid arthritis have a somewhat higher incidence of positive reactions to S.E.A. tests in both initial and multiple determinations than females.

Limitation of the diagnosis of rheumatoid arthritis to individuals with rheumatoid nodules results in 95 per cent positive reactions to the S.E.A. test and 92 per cent positive reactions to the Fii test on first determinations.

The serum titers of the majority of patients with rheumatoid arthritis vary within two dilution tubes for either test during the two years following the initial test. The response of these patients to any of the usual modes of therapy does not affect the reactions in these tests. The S.E.A. test has a greater tendency to convert from positive to negative or vice versa than does the Fii test.

Joint fluid studied concurrently with serum usually has the same or a significantly higher titer.

The mechanisms of these tests and the nature of the circulating rheumatoid factor have been discussed.

REFERENCES

1. Waaler, E. On the occurrence of a factor in human serum activating the specific agglutination of sheep

blood corpuscles. *Acta path. et microbiol. Scandinav.*, 17: 172, 1940.

2. Rose, H. M., Ragan, C., Pearce, E. and Lipman, M. D. Differential agglutination of normal and sensitized sheep erythrocytes by sera of patients with rheumatoid arthritis. *Proc. Soc. Exper. Biol. & Med.*, 68: 1, 1948.
3. Heller, G., Jacobson, A. S. and Kolodny, M. H. A modification of the hemagglutination test for rheumatoid arthritis. *Proc. Soc. Exper. Biol. & Med.*, 72: 316, 1949.
4. Heller, G., Jacobson, A. S., Kolodny, M. H. and Schuman, R. L. The hemagglutination test for rheumatoid arthritis. I. An immunological analysis of the factors involved in the reaction. *J. Immunol.*, 69: 27, 1952.
5. Jacobson, A. S., Kammerer, W. H., Kolodny, M. H. and Heller, G. The hemagglutination test for rheumatoid arthritis. A clinical analysis. *Ann. Rheumat. Dis.*, 12: 321, 1953.
6. Svartz, N. and Schlossman, K. The agglutinating factor for sensitized sheep erythrocytes in serum and joint fluids from rheumatoid arthritis patients. *Ann. Rheumat. Dis.*, 9: 377, 1950.
7. Svartz, N. and Schlossman, K. Agglutination of sensitized sheep precipitable serum substances in rheumatoid arthritis. *Acta med. Scandinav.*, 146: 313, 1953.
8. Heller, G., Jacobson, A. S., Kolodny, M. H. and Kammerer, W. H. The hemagglutination test for rheumatoid arthritis. II. The influence of human plasma fraction II (gamma globulin) on the reaction. *J. Immunol.*, 72: 66, 1954.
9. Ziff, M., Brown, P. and McEwen, C. Hemagglutination tests for rheumatoid arthritis with enhanced sensitivity using the euglobulin fraction. *Bull. Rheumat. Dis.*, 5: 75, 1954.
10. Robinson, W. D., Boland, E. W., Bunim, J. J., Crain, D. C., Engleman, E. P., Graham, W., Lockie, L. M., Montgomery, M. M., Ragan, C., Ropes, M. W., Rosenberg, E. F. and Smyth, C. J. Rheumatism and arthritis (tenth rheumatism review). *Ann. Int. Med.*, 39: 497, 1953.
11. Steinbrocker, O., Traeger, C. H. and Batterman, R. C. Therapeutic criteria in rheumatoid arthritis. *J. A. M. A.*, 140: 659, 1949.
12. Ball, J. Serum factor in rheumatoid arthritis agglutinating sensitized sheep red cells. *Lancet*, 2: 520, 1950.
13. Heller, G., Kolodny, M. H., Lepow, I. H., Jacobson, A. S., R vera, M. E. and Marks, G. H. The hemagglutination test for rheumatoid arthritis. IV. Characterization of the rheumatoid agglutinating factors by analysis of serum fractions prepared by ethanol fractionation. *J. Immunol.*, 74: 340, 1955.
14. Pike, R. M., Sulkin, S. E. and Coggeshall, H. C. Serological reactions in rheumatoid arthritis. II. Concerning the nature of the factor in rheumatoid arthritis serum responsible for increased agglutination of sensitized sheep erythrocytes. *J. Immunol.*, 63: 447, 1949.

Agglutination and Inhibition by Serum Globulin in the Sensitized Sheep Cell Agglutination Reaction in Rheumatoid Arthritis*

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THE accessory agglutinating factor present in the serum of patients with rheumatoid arthritis was first demonstrated by Waaler in 1940.[1] Rose, Ragan, Pearce and Lipman,[2] comparing the agglutination of sensitized and unsensitized cells, found that 37 per cent of patients with rheumatoid arthritis and 4 per cent of control subjects showed a differential titer of 16 or higher. Modification of this test by preliminary removal of heterophil antibody with sheep red blood cells[3] yielded positive results in 67 per cent of patients with rheumatoid arthritis. In a later test described by Heller, Jacobson, Kolodny and Schuman,[4] based on the capacity of sheep serum to potentiate the action of the accessory agglutinating factor, 85 per cent of the serums from patients with peripheral rheumatoid arthritis and 1.6 per cent of the serums from control subjects yielded positive results. Svartz and Schlossmann in a recent report[5] have described a cold globulin fraction from serum which yielded positive results in 95 per cent of cases of rheumatoid arthritis, and in 2 per cent of patients with other diseases.

During the course of the present studies, in which an inhibitor of sensitized sheep cell agglutination is described, the presence of such an agent was independently described by Heller, Jacobson, Kolodny and Kammerer[6] in Cohn fraction II of serum.

Previous attempts to raise the sensitivity of the Waaler-Rose agglutination test in whole serum by addition of increased amounts of amboceptor have been accompanied with a corresponding decrease in specificity. In seeking to explain the high percentage of negative reactions to agglutination tests in patients with rheumatoid arthritis, the possible presence in serum of an inhibitor of sensitized sheep cell agglutination was considered, and a variety of serums were tested for inhibitory activity. Inhibition was observed in twenty-three of sixty-two non-rheumatoid whole serums. The demonstration of inhibition by whole serum suggested the possibility of increasing the sensitivity of the agglutination reaction by separation of the inhibitor from the agglutinating factor. Partial separation was achieved through precipitation of the euglobulin fraction by dialysis against dilute phosphate buffer at pH 6. When the agglutinating activity of the euglobulin fraction was tested, over 90 per cent of rheumatoid serums yielded positive results, and there were from 2 to 7 per cent positive reactions among control subjects. When the capacity of the euglobulin fraction to inhibit agglutination produced by known positive serum was investigated, it was found that 100 per cent of rheumatoid serums failed to inhibit whereas only 4 per cent of control serums behaved in a similar manner.

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EXPERIMENTAL TESTS

Patients. During the first year of this study a total of 105 patients with rheumatoid arthritis, including seven with the juvenile form, and 104 control subjects were studied. The control subjects included fourteen normal persons, four pregnant women, three patients with psychogenic rheumatism, and one each with the following: false positive Wassermann reaction, chronic urticaria, sprain, ulcerative colitis and acromegaly. The remainder of the control subjects were drawn from a variety of rheumatic diseases consisting of the following: gout, eleven; active disseminated lupus erythematosus, seven; rheumatoid spondylitis, ten; psoriatic arthritis, six; rheumatic fever, nineteen; inactive rheumatic heart disease, six; degenerative joint disease, eleven; myositis, two; dermatomyositis, one; diffuse scleroderma, one; fibrositis, two; and undiagnosed monarticular arthritis, one. All patients were examined by the authors; the diagnosis of rheumatoid arthritis was based in almost all cases on periods of observation ranging from months to years. Sixty per cent of the patients with rheumatoid arthritis were women, 40 per cent were men. Of the eighty-three adult rheumatoid arthritis serums that were fractionated, sixty-three were from patients with active rheumatoid arthritis, thirteen from patients with low grade activity, and seven from patients who were considered inactive.

A second series consisting of fifty patients with adult rheumatoid arthritis and fifty-eight control subjects was subsequently studied by different observers. The control group was comprised of eight normal individuals; twenty-six patients awaiting elective surgery; five with ankylosing spondylitis; two with osteoarthritis; four with rheumatic fever; one each with tuberculous arthritis, psoriatic arthritis, fibrositis and scleroderma; and nine patients with miscellaneous diseases. Twenty-five of the patients with rheumatoid arthritis and twelve of the control subjects of the second series are also included in the first series. Thus a total of 133 tests were done on the serums of 108 patients with rheumatoid arthritis, and 162 tests on the serums of 150 control subjects in both series.

Sheep cells were obtained from the slaughterhouse at weekly intervals, diluted in an equal volume of Alsever's solution[7] and stored at 4°C. Before use the cells were washed three times in physiologic saline solution.

Rabbit anti-sheep cell serum (amboceptor) containing 50 per cent glycerine* was made up as required in a 0.2 per cent phenol in saline solution to a dilution of 1/100; this was stored at 4°C.

Agglutination Procedure. Test serums were inactivated by heating at 56°C. for one-half hour. They were then twice absorbed (undiluted) with an equal volume of packed sheep red blood cells for one hour at 37°C. The absorbed serums were then tested against unsensitized cells to insure complete removal of the heterophil antibody.

Agglutination was carried out with both whole serum and globulin fractions by the following method, which is a modification of that described by Hobson and Gorill:[8] to 0.25 ml. of amboceptor in dilutions of 1/100, 1/200, 1/300, etc., 0.5 ml. of saline solution and 0.25 ml. of a 3 per cent red cell suspension were added. The final volume was 1 ml. The end point of agglutination, read after storing the tubes overnight in the refrigerator, was considered to be the basic agglutinating titer of the red cells.

The red cell suspension was sensitized with an equal volume of a solution containing one-half of the basic agglutinating titer, allowing it to stand at 37°C. for thirty minutes; 0.5 ml. of the sensitized cell suspension was added to an equal volume of serial twofold dilutions of either inactivated and absorbed test serums, cold globulin or euglobulin fractions, beginning with an initial dilution of 1/14. The tubes were stored in the refrigerator overnight and read by agitation against a strong light. Agglutination occurring in dilutions of 1/28 or over was considered positive in the first series, and 1/14 or greater in the second series.

Whole serum agglutination was also tested by the method of Heller, Jacobson, Kolodny and Schuman.[4]

Cold globulin fraction was separated by the method of Svartz and Schlossmann.[5] Testing was carried out as already described for the euglobulin fraction and, for comparison, the same criteria for a positive test were employed. These are not strictly comparable to the test conditions described by Svartz and Schlossmann.

Euglobulin Fractionation. One to 3 ml. of inactivated and absorbed serum were diluted with an equal volume of distilled water and dialyzed in the cold against 6 L. of M/150 citrate-phosphate buffer of pH 5.8† for two

* Obtained from Dr. G. Heller and later from Sharp and Dohme, Philadelphia, Pa.

† Two hundred milliliters of 0.2 M disodium phos-

days with occasional agitation. No more than twelve samples of serum were dialyzed in the same bath, which was wide enough to avoid crowding of the dialysis bags. The precipitate was washed once with 1.5 ml. of chilled buffer, dissolved in a volume of M/150 citrate-phosphate buffer in isotonic saline at pH 7.0 equivalent to the volume of serum taken for dialysis, and tested for agglutination. In some cases the supernatant solution was similarly tested after addition of an equal volume of 1.8 per cent saline solution. The neutral buffer was prepared by adding 25.8 ml. of 0.1M citric acid to 100 ml. of 0.2M disodium phosphate and making up to 3 L. with isotonic saline.

Test for Inhibition. To a fixed amount of positive-reacting rheumatoid serum, serial dilutions of the euglobulin fraction to be tested were added. The rheumatoid serums used had titers of 1/448 to 1/1792. The sensitized cell suspension was made up by addition of an equal volume of rabbit antiserum diluted to contain one-twentieth of the basic agglutinating titer to 0.5 per cent sheep cells as described by Heller and co-workers.[4] The final volume in each tube was 1 ml., consisting of 0.25 ml. of serially diluted euglobulin, 0.25 ml. of appropriately diluted positive rheumatoid serum (v. seq.), and 0.5 ml. of sensitized cell suspension. The dilution of euglobulin in the first tube, before addition of the cell suspension, was 1/14 (i.e., 0.25 ml. of 1/7 euglobulin and 0.25 ml. of positive rheumatoid serum diluted as to be described). Subsequent tubes contained serial twofold dilutions of euglobulin.

The rheumatoid serum to be inhibited was prepared as follows: 0.5 ml. of serum was doubly diluted in saline solution and 0.5 ml. of the sensitized cell suspension prepared as described in the foregoing paragraph containing 1/20 of the basic agglutinating dose of amboceptor was added to each dilution. The end point of agglutination was noted and 0.25 ml. of the dilution corresponding to 1/8 of this end-point titer (i.e., containing eight "units" of agglutinating activity) was added to each tube of the test system for inhibition as described. One control tube containing 0.5 ml. of sensitized cells and 0.5 ml. saline solution, and another containing 0.5 ml. of sensitized cells, 0.25 ml. of diluted positive serum, and 0.25 ml. of saline solution were prepared. The inhibition titer was expressed as the highest dilution of euglobulin in the presence of which agglutination did not occur. This represents the euglobulin dilution which effects an eightfold decrease in agglutination titer of rheumatoid serum. Inhibition is recorded as being present when observed at a euglobulin dilution of 1/14.

All readings were made using a hand lens, by agitation of the tubes in front of direct light, after allowing the tubes to remain overnight in the refrigerator.

Determination of the presence or absence of inhibition of agglutination has been a difficult technical procedure requiring considerable experience in reading agglutination tests. It has been possible, however, for all of five observers who have read these tests to obtain consistent results.

Albumin and globulin concentrations were determined by the method of Albanese, Irby and Saur.[9]

RESULTS

Table 1A presents the distribution of agglutination titers using the euglobulin fraction in eighty-three patients with adult rheumatoid arthritis and 104 control subjects of the first series. It is seen that 91.6 per cent of rheumatoid subjects had titers of 1/28 or greater whereas only 1.9 per cent of control individuals fell into the same range. On the basis of these results a titer of 1/28 or above is referred to in this report as "positive" and of 1/14 or less as "negative" in the first series.

The distribution of agglutination titers using the euglobulin fraction in the second series of subjects is shown in Table 1B. For the second group of observers it appeared that an agglutination titer of 1/14 rather than 1/28 could be considered as a positive test since, as is shown in Table 1B, 92 per cent of patients with rheumatoid arthritis had a titer of 1/14 or higher whereas only 7 per cent of control subjects fell into this range. It would appear that there was a one-tube difference in the reading of the end point of agglutination between the two groups of observers both with respect to the rheumatoid and control subjects.

In Table 11 the results obtained with the euglobulin fractions (first series) are compared with those obtained using the cold globulin

phate and1 50 ml. of 0.1 M citric acid were diluted to 6 L. The pH was adjusted to 5.8 by addition of small amounts of the disodium phosphate solution or citric acid solution. The final pH of the serum samples was approximately 6.0.

TABLE I

AGGLUTINATION TITERS OF EUGLOBULIN FRACTIONS IN RHEUMATOID AND CONTROL SUBJECTS

	No Agglutination at 1/14 (% of Patients)	Agglutination Titer (% of Patients)						
		1/14	1/28	1/56	1/112	1/224	1/448	Total
A. First Series								
Adults with rheumatoid arthritis (83)	6.0	2.4	14.4	19.4	30.2	16.8	10.8	91.6
Control subjects (104)	78.8	19.3	0	0	0	1.9	0	1.9
B. Second Series								
Adults with rheumatoid arthritis (50)	8	16	20	18	16	12	10	92
Control subjects (58)	93	5	0	0	0	2	0	7

TABLE II

AGGLUTINATION AND INHIBITION BY WHOLE SERUM AND GLOBULIN FRACTIONS OF SUBJECTS WITH RHEUMATOID ARTHRITIS AND THOSE IN THE CONTROL GROUP

		Euglobulin		Cold Globulin	Whole Serum	
		Agglutination *	Inhibition Present	Agglutination *	Agglutination *	Agglutination †
Adults with rheumatoid arthritis	% positive	92	0	75§	78	65
	No. patients	83‡	83	63	103‡	86
Control subjects	% positive	2**	96	3§	13	5
	No. patients	104	104	75	104	90

* Tested by the modified Waaler-Rose method described (see Experimental Tests.)

† By the method of Heller, Jacobson, Kolodny and Schuman.[4]

‡ In eighty patients euglobulin and whole serum were tested simultaneously. Agglutination was observed in 90 per cent with the euglobulin fraction and in 78 per cent with whole serum.

§ Eighty per cent of rheumatoid subjects and 5 per cent of control subjects showed agglutination in a titer of 1/14.

** One patient had subacute disseminated lupus erythematosus and a second had advanced degenerative joint disease.

fraction and the two whole serum tests employed, namely, the modified Waaler-Rose test herein described and the procedure of Heller, Jacobson, Kolodny and Schuman.[4] As mentioned, 92 per cent of the adult group with rheumatoid arthritis tested showed positive results to the agglutination tests with the euglobulin fraction while only 2 per cent of controls gave positive results. Sixty-five and 78 per cent of rheumatoid serums, respectively, gave positive results with the two whole serum tests, and 75 per cent with the cold globulin fraction; false positive reactions were observed in 3 to 18 per cent of control subjects by these three tests. Since the same group of patients was included in all tests (except that some test groups were somewhat larger than others), it would appear that the use of the euglobulin fraction provides a more specific and more sensitive test than hitherto available.

Results of the test with whole serum were most frequently negative when the disease was

TABLE III

RELATION OF WHOLE SERUM AND EUGLOBULIN AGGLUTINATION TESTS TO ACTIVITY OF RHEUMATOID ARTHRITIS

Pattern of Tests	Whole Serum	Euglobulin		Number of Patients		
	Agglutination*	Agglutination	Inhibition	Active	Low Grade Activity	Inactive
1	+	+	0	55	10	3
2	0	+	0	5	3	0
3	0	0	0	3	0	4
4	0	0	+	0	0	0
			Total	63	13	7

* Included here are positive results with either whole serum test.

considered inactive. In seven patients in whom the modified Waaler-Rose test on whole serum gave positive results during the period of observation, the euglobulin fraction was already positive in four and became positive at the same time as the whole serum in three. In these cases the last values obtained were tabulated, which would tend to favor the results of the whole serum test since there were no other instances of conversion of the euglobulin from negative to positive. There was only one instance of reversal of a positive reaction to the euglobulin test.

When the capacity of the euglobulin fraction to inhibit agglutination by known positive serum was determined, no rheumatoid serum showed inhibition at any stage of the disease represented in this series, whereas 96 per cent of control serums did inhibit agglutination. (Table III.) In the second series described previously, all of forty-six serums tested of patients with rheumatoid arthritis, of which twenty-four were also tested on the first series, yielded euglobulin fractions which failed to inhibit known positive serum, whereas the euglobulin fraction of all of thirty-eight control subjects did produce inhibition. This confirms the results obtained in the first series. In the sense that failure of the euglobulin fraction to inhibit known positive serum appears to be characteristic of rheumatoid arthritis, such a result will be considered positive.

Agglutination tests were carried out in seven patients with juvenile rheumatoid arthritis, of whom one was seven years of age; two, twelve; one, thirteen; and three, eighteen. In all cases the onset of the disease had occurred before the age of twelve. The direct euglobulin agglutination titer was positive in five of these patients, and in all cases the euglobulin failed to inhibit the agglutinating activity of known positive serum.

In adult patients who had low grade activity (Table III) results were similar to those obtained in active cases but patients who were considered to be inactive had negative results in tests with both whole serum and euglobulin in four of seven cases. In all cases, however, the euglobulin failed to inhibit the accessory agglutination activity of known positive serum.

Thirteen of fourteen patients with rheumatoid arthritis in the entire group who had had the disease for less than a year gave positive reactions to tests directly with the euglobulin fraction. Eight of the thirteen patients with positive reactions were tested in the first series and gave titers of 1/28 or higher. Of the five patients who were tested in the second series, two showed titers of 1/14, one a titer of 1/28, and two, titers of 1/112.

All of ten patients who had had the disease for six months or less had positive reactions, in one case within three weeks of onset, in one within one month, and in two within two months.

Whole serum from three patients of seven with disseminated lupus erythematosus (Table IV) showed agglutination, but in only one of these was the euglobulin fraction active. The euglobulin fraction from this patient, who had

TABLE IV

RESULTS OF WHOLE SERUM AND EUGLOBULIN TESTS IN DISSEMINATED LUPUS ERYTHEMATOSUS, RHEUMATOID SPONDYLITIS AND PSORIATIC ARTHRITIS

Pattern of Tests	Whole Serum	Euglobulin		Number of Cases		
	Agglutination*	Agglutination	Inhibition	Disseminated L.E.	Rheumatoid Spondylitis†	Psoriatic Arthritis‡
1	+	+	0	1	0	0
2	+	0	+	2	2	0
3	0	0	+	4	8	6
			Total	7	10	6

* Included here are positive results by either whole serum test.

† Five had peripheral joint involvement.

‡ Three had psoriatic arthropathy; one had rheumatoid arthritis with psoriasis, and in two the diagnosis was uncertain.

chronic joint involvement, also failed to inhibit.* Thus the patient exhibited the pattern seen in rheumatoid arthritis, both clinically and serologically. In the other two positive serums the agglutinating factor had a solubility differing from that of the rheumatoid factor in not precipitating in the euglobulin fraction.

Of ten patients with rheumatoid spondylitis two had positive reactions in whole serum but the euglobulin fraction when tested directly gave a negative test and inhibited in all instances.† Serums from all six patients with psoriasis and arthritis (see Table IV) were negative in all tests.

In testing serum fractions for agglutination and inhibition five different patterns were observed. (Table V.) In the pattern seen most commonly in patients with rheumatoid arthritis, both the whole serum and the euglobulin showed agglutinating activity and the euglobulin failed to inhibit known positive serum (Pattern 1). Inhibition appeared in the supernatant following precipitation of the euglobulin fraction. The second pattern, also seen in rheumatoid serums (Pattern 2), showed a negative reaction in the whole serum but a positive reaction in the euglobulin fraction with partition of at least a portion of the inhibitor into the supernatant solution on precipitation of the euglobulin fraction. Pattern 3, which was characteristic of the usual negative serums, demonstrated here by a patient with gout, showed no agglutinating activity in any fraction but did show inhibitory activity in whole serum, euglobulin and supernatant fractions. Whole serum of pattern 4, as exemplified in the table by a patient with disseminated lupus erythematosus, produced agglutination while the euglobulin fraction gave no agglutination and was strongly inhibitory. The supernatant from the euglobulin, however, contained the agglutinating activity. A fifth pattern was noted in a single patient with degenerative joint disease who showed a very high titer of inhibition in whole serum and in the euglobulin fraction but the euglobulin supernatant had agglutinating activity.

The observation that the euglobulin fraction of non-rheumatoid serums contained an inhibitor suggested assay of the inhibitory activity of a sample of pooled human gamma globulin,* and inhibition was found to be present in a titer

* In a group of seventeen patients with disseminated lupus erythematosus recently studied, four have shown clinical features of both disseminated lupus erythematosus and rheumatoid arthritis. All four of these patients, like the one mentioned, have shown positive L.E. cells as well as positive sheep cell agglutination titers in the euglobulin fraction.

† A positive test with the euglobulin fraction has recently been obtained in a patient with juvenile rheumatoid arthritis, peripheral joint involvement and spondylitis. In this patient the onset of the disease began in the peripheral joints.

* Human gamma globulin was obtained from the New York City Department of Health.

TABLE V

PATTERNS OF AGGLUTINATION AND INHIBITION TITERS OF WHOLE SERUM, EUGLOBULIN AND EUGLOBULIN SUPERNATANT FRACTIONS IN SERUMS OF INDIVIDUAL PATIENTS WITH VARIOUS DISEASES

Pattern	Whole Serum Test	Disease	Whole Serum Titers		Euglobulin Titers		Euglobulin Supernatant Titers	
			Agglutination	Inhibition	Agglutination	Inhibition	Agglutination	Inhibition
1	pos.	rheumatoid arthritis (N. B. ♀)	112	0	224	0	0	70
2	neg.	rheumatoid arthritis (N. E. ♀)	0	56	40	0	0	64
3	neg.	gout (C. H. ♂)	0	112	0	80	0	64
4	pos.	disseminated lupus erythematous (A. M. ♀)	56	0	0	320	56	0
5	neg.	osteoarthritis (L. G. ♀)	0	14,000	0	20,000	448	0

TABLE VI

EFFECT OF POOLED HUMAN GAMMA GLOBULIN ON SENSITIZED SHEEP CELL AGGLUTINATION IN A PATIENT WITH RHEUMATOID ARTHRITIS

Date	Agglutination Titers				Inhibition by Euglobulin	Serum Albumin-Globulin Concentration (gm. %)
	Whole Serum †	Whole Serum ‡	Euglobulin	Cold Globulin		
Feb. 2	28/112	112	112	56	0	.....
Feb. 23*	28/112	112	112	28	0	3.5/4.1
Feb. 23 (2 hr.)	28/56	56	0	28	0	3.5/3.9
Feb. 23 (6 hr.)	14/28	28	28	14	0	.....
Feb. 24*	28/56	28	14	0	14	3.3/4.4
Feb. 25*	0/0	14	0	0	14	2.8/4.1
Feb. 26	14/0	0	0	..	112	3.6/4.6
Feb. 27	0/0	28	28	56	0	.....
March 1	28/112	112	224	56	0	.....

* Ten milliliters of pooled human gamma globulin containing 165 ± 15 mg. per ml. injected intramuscularly; titers determined prior to injection on days noted.

† By the method of Heller, Jacobson, Kolodny and Schuman.[4]

‡ Tested by the method described (see Experimental Tests.)

of 1/1729. It was then decided to determine whether parenteral administration of gamma globulin to a patient with rheumatoid arthritis, who gave a positive serum agglutination reaction, would decrease the titer.

Ten milliliters of pooled human gamma globulin containing 165 mg. of protein per milliliter were injected intramuscularly on three successive days. The data in Table VI demonstrate that there was a diminution in the agglutinating activity of whole serum and of the euglobulin fraction within two hours after the first injection. At the end of two days all agglutination tests gave negative results and the euglobulin fraction had developed inhibitory activity. Two days after the last injection, however, all tests

but one had become positive again. Serum albumin and globulin concentrations showed no significant change. There was no observable change in the patient's condition during this time.

When serum from a patient with rheumatoid spondylitis and another with psoriatic arthritis were fractionated into albumin and globulin by precipitation with 50 per cent ammonium sulfate, all the inhibitory activity of the serum could be recovered in the globulin fraction, while the albumin was inactive. (Table VII.)

TABLE VII
AMMONIUM SULFATE FRACTIONATION OF SERUM INHIBITOR IN TWO SERUMS GIVING NEGATIVE REACTIONS

Patient	Condition	Inhibition Titer		
		Whole Serum	Albumin	Globulin
K. B. ♂	Rheumatoid spondylitis	112	0	112
H. G. ♀	Psoriatic arthritis	112	0	112

COMMENTS

The data suggest that the occurrence of agglutination of sensitized sheep cells either by whole serum or by serum globulin of patients with rheumatoid arthritis may be determined under given conditions by the presence not only of an agglutinating factor but also of an inhibitor. That inhibitor is indeed present in rheumatoid serum is demonstrated (patterns 1 and 2, Table V) by the inhibitory activity of the supernatant fraction from such serum after precipitation of the euglobulin. The quantitative effect of the inhibitor on the titer of the agglutination reaction, if any, under the test conditions described remains unknown, nor is it known whether the inhibitor is a single substance or a group of non-specific substances.

Since both the euglobulin and its supernatant fraction from negative serums showed inhibition, it is likely that precipitation of the euglobulin fraction of rheumatoid serums separates only part of the inhibitor from the agglutinator. The increase in the percentage of positive tests from 78 per cent in whole serum (Table II) to 92 per cent using the euglobulin fraction, if significant, may be related to removal of inhibitor, but there is no direct evidence that this is true. A more important advantage to be derived from the use of the euglobulin fraction lies in the fact that the incidence of false positive reactions fell from 13 per cent to 2 per cent under the same conditions in the same group of patients. It would appear probable, also, that the inability of the euglobulin fraction of all rheumatoid serums to inhibit known positive serum is merely a more sensitive indication of the presence of agglutinating factor, the latter presumably being present in negatively reacting euglobulin fractions in amounts insufficient to cause agglutination directly. It should be pointed out that the test systems employed for titration of inhibitor and agglutinator were different and that the titers given are therefore not comparable.

Contrary to experience with earlier attempts to increase the sensitivity of the test by addition of larger amounts of amboceptor, the modification here reported does not increase the number of false positive reactions. The relatively high value of 78 per cent of positive results in rheumatoid patients by the whole serum test described is no doubt due to the fact that sensitization was carried out with the relatively high titer of one-half the basic agglutinating dose of amboceptor. While this increased the percentage of positive results in rheumatoid subjects above those previously reported,[3] it also increased the incidence of false positive reactions to 13 per cent. When the euglobulin fraction was tested by the same technic, using the same amount of amboceptor, there was not only a rise in the percentage of positive results but also a decrease in the percentage of false positive reactions. The latter change may be due to the removal of non-specific agglutinins.

Observations not reported here in detail showed no striking increase in the titer of the euglobulin fraction of rheumatoid serums over that of the whole serum. It is possible that the activity of the inhibitor is small when compared with that of the agglutinator. Further evaluation of this question must await isolation of agglutinator and inhibitor in sufficient purity to allow comparison of their relative activities.

Svartz and Schlossmann[5] have reported that 95 per cent of patients with rheumatoid arthritis gave positive tests with a cold precipitable globulin fraction. Using this fraction, prepared

from 0.5 ml. of serum and testing for agglutination by the procedure herein described, 75 per cent of the group of rheumatoid patients tested had positive reactions. Thus when tested under the same conditions, the euglobulin fraction was more often positive than the cold globulin. It should be noted that the conditions of sensitization employed in this investigation were not the same as those of Svartz and Schlossmann.[5]

Three patients of seven with disseminated lupus erythematosus gave positive results on at least one test. (Table IV.) This confirms previous experience.[8,10] Six of seven patients, however, gave negative results in tests with euglobulin; the seventh patient, in whom the diagnosis of lupus erythematosus was based on the finding of a positive L.E. test, showed chronic joint involvement resembling that seen in rheumatoid arthritis. In two cases the euglobulin fraction was negative while the whole serum was positive. This was not observed in any rheumatoid serum tested. It would thus appear that the agglutinating factor present in some cases of lupus erythematosus may on occasion have a different solubility in dilute electrolyte solutions from that of the factor occurring in rheumatoid arthritis, although subsequent experience indicates that this is rare.

Eight of ten patients with rheumatoid spondylitis gave negative results in all three tests and also gave negative results when the euglobulin fraction was tested. This was true in spite of the fact that five of the patients had peripheral joint involvement, other than hips and shoulders. The complete absence of positive tests with the euglobulin fraction even on the basis of the inhibition procedure which showed no inhibition in 100 per cent of patients with peripheral rheumatoid arthritis, strongly supports other evidence suggesting a fundamental difference between the two diseases.

All six patients with psoriasis and arthritis had negative reactions to all tests. Three of the six were considered to have psoriatic arthropathy,[11] one psoriasis with rheumatoid arthritis, and in two patients the diagnosis was uncertain.

The negative nature of the agglutination test in psoriatic arthritis and in rheumatoid spondylitis has been repeatedly pointed out.[3,10,12] In view of the apparent similarity of the peripheral joint involvement in so-called rheumatoid spondylitis and many cases of "psoriatic arthritis" with that of peripheral rheumatoid arthritis, both pathologically[13] and clinically, the absence of agglutinating factor in these diseases is impressive. It suggests that the agglutinating factor is not likely to be a non-specific by-product of chronic synovial inflammation but may possibly be related to the underlying mechanism of the disease, and that these joint diseases, often thought to be merely variants of rheumatoid arthritis, are distinct entities. In view of the fact that the diagnosis in one of the psoriatic cases was considered to be rheumatoid arthritis with coexistent psoriasis rather than "psoriatic arthropathy," it is also possible that the presence of psoriasis in some way alters the reaction.

It has been commonly found that the agglutination reaction has tended to be negative early in the course of rheumatoid arthritis. Brown, Bunim and McEwen[14] obtained positive reactions in only 35 per cent of patients who had the disease less than one year. All ten patients of the present group who had had symptoms for six months or less showed tests characteristic of rheumatoid arthritis with both the euglobulin agglutination and inhibition tests. This also points to an essential relationship between the agglutinating factor and the rheumatoid process.

Previous studies[15] have demonstrated positive agglutination tests in only a small minority of serums of patients with juvenile rheumatoid arthritis. Utilizing the euglobulin fraction, five of seven patients showed agglutination and all failed to show inhibition. Thus it is likely that the difficulty in demonstrating the agglutinating factor in the juvenile form may be merely a quantitative one.

It was possible to block the agglutination test in a patient's serum by intramuscular administration of gamma globulin. (Table VI.) The effect, however, was transitory since, two days after the last injection, inhibitory activity was no longer present in the euglobulin fraction and the results of the agglutination tests had again reverted to positive. These results indicate that the injected inhibitor was either rapidly inactivated or disappeared quickly from the circulation.

SUMMARY

1. When the euglobulin fraction of serum was employed in the sensitized sheep cell agglutination reaction, over 90 per cent of 133 tests in 108 patients with rheumatoid arthritis gave positive results. Two to 7 per cent of 162 tests in 150 control subjects gave positive reactions.

2. Inhibition of agglutination by a variety of serums is demonstrated, and a procedure is described for measurement of inhibition of positive rheumatoid serum by the euglobulin fraction. The serum euglobulin of 105 patients with rheumatoid arthritis failed to show inhibition in 100 per cent of the cases whereas over 96 per cent of the euglobulin fractions of control subjects caused inhibition.

3. A positive agglutination test in the serum of a patient with rheumatoid arthritis became temporarily negative soon after intramuscular administration of pooled human gamma globulin.

4. The sensitized sheep cell agglutinating factor was demonstrated in the euglobulin fraction in five of seven patients with juvenile rheumatoid arthritis. In no case of adult rheumatoid spondylitis or arthritis with psoriasis tested was agglutination observed.

Acknowledgment: We wish to thank Dr. Lars Boettiger for his help in this work.

REFERENCES

1. Waaler, E. On the occurrence of a factor in human serum activating the specific agglutination of sheep blood corpuscles. *Acta path. et microbiol. Scandinav.*, 17: 172, 1940.
2. Rose, H. M., Ragan, C., Pearce, E. and Lipman, M. O. Differential agglutination of normal and sensitized sheep erythrocytes by sera of patients with rheumatoid arthritis. *Proc. Soc. Exper. Biol. & Med.*, 68: 1, 1948.
3. Heller, G., Jacobson, A. S. and Kolodny, M. H. A modification of the hemagglutination test for rheumatoid arthritis. *Proc. Soc. Exper. Biol. & Med.*, 72: 316, 1949
4. Heller, G., Jacobson, A. S., Kolodny, M. H. and Schuman, R. L. The hemagglutination test for rheumatoid arthritis. I. An immunological analysis of the factors involved in the reaction. *J. Immunol.*, 69: 27, 1952.
5. Svartz, N. and Schlossmann, K. A serum cold precipitable hemagglutinating factor in rheumatoid arthritis. *Acta med. Scandinav.*, 149: 83, 1954.
6. Heller, G., Jacobson, A. S., Kolodny, M. H. and Kammerer, W. H. The hemagglutination test of rheumatoid arthritis. II. The influence of human plasma fraction II (gamma globulin) on the reaction. *J. Immunol.*, 72: 66, 1954.
7. Bukantz, S. C., Rein, C. R. and Kent, J. F. Studies in complement fixation. II. Preservation of sheep's blood in citrate-dextrose mixtures (modified Alsever's solution) for use in the complement fixation reaction. *J. Lab. & Clin. Med.*, 31: 394, 1946.
8. Hobson, D. and Gorill, R. H. Agglutination test for rheumatoid arthritis. *Lancet*, 262: 389, 1952.
9. Albanese, A. A., Irby, V. and Saur, B. The colorimetric estimation of proteins in various body fluids. *J. Biol. Chem.*, 166: 231, 1946.
10. Ball, J. Sheep cell agglutination test for rheumatoid arthritis, a clinico-pathological study. *Ann. Rheumat. Dis.*, 11: 97, 1952.
11. Hench, P. S., Bauer, W., Ghrist, D., Hall, R. Holbrook, W. P., Key, J. A. and Slocumb, C. The present status of rheumatism and arthritis: review of American and English literature for 1936 (fourth rheumatism review). *Ann. Int. Med.*, 11: 1089, 1938.
12. Alexander, R. and deForest, G. The sensitized sheep cell agglutination reaction in rheumatoid arthritis. *Am. J. Med.*, 16: 191, 1954.
13. Collins, D. H. Pathology of Articular and Spinal Diseases, pp. 203 and 321. London, 1949. Edward Arnold & Co.
14. Brown, R., Bunim, J. J. and McEwen, C. Differential sheep cell agglutination test in rheumatoid arthritis. *Ann. Rheumat. Dis.*, 8: 299, 1949.
15. Ball, J. Serum factor in rheumatoid arthritis agglutinating sensitized sheep red cells. *Lancet*, 2: 520, 1950.

Synovial Specimens Obtained by Knee Joint Punch Biopsy*

Histologic Study in Joint Diseases

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IN 1932 Forestier[1] described a method for obtaining synovial tissue by the use of a dental nerve extractor introduced into the joint space through a large caliber, hollow needle. Using this procedure he obtained a number of biopsy specimens, during the following two or three years but did not publish the results.[2] In 1951, in the first of several articles and abstracts, Polley and Bickel[3–5] reported the development and extensive use of a punch biopsy procedure in joint diseases. Considerable interest has been aroused in the method as a practical means of obtaining, with greater frequency than had previously been considered justifiable, specimens of synovial membrane as an aid in the differential diagnosis of joint diseases. In the present study the knee joint punch biopsy was employed as a diagnostic aid in patients with joint disease when the diagnosis was uncertain, and also as an investigative method when a study of synovial membrane was thought to be valuable in defining the morphologic characteristics of the synovium.

METHOD

The instrument and technic used were those developed by Polley and Bickel.[4] The procedure, except in the case of two children who received a general anesthetic, was performed in the clinic treatment room rather than in the operating room as described by Polley and Bickel. The knee is prepared and draped in accordance with standard surgical practice, using a six-minute phisoderm® scrub, merthiolate,® or 2% tincture of iodine, followed by 50% alcohol. The surgeon is masked as are the nurse and patient and separate pairs of sterile gloves are employed for preparing the patient and for the instrumentation. One and a half per cent procaine solution is injected into the skin and about the underlying structures, particular care being taken to infiltrate the joint capsule widely in the path of the instrument. The knee joint is approached through the suprapatellar bursa. (Fig. 1.) Synovial fluid is aspirated through the hollow needle and examined as described by Robinson, Duff and Smith.[6] About four specimens of synovium are obtained from the perimeters of the joint space and are fixed immediately in 10 per cent formalin. Immediately after the procedure patients are permitted to walk but are asked to avoid unnecessary activity for the following twenty-four hour period. The specimens are prepared in a routine manner and stained with hematoxylin and eosin.

STANDARDS

For interpretation of the joint fluid characteristics, the normal values reported by Ropes and Bauer[7] have been used. (Table I.) The classification of pathologic fluids into three groups by

TABLE I

NORMAL SYNOVIAL FLUID VALUES (ROPES AND BAUER[7])

Cytology:	
Leukocytes per cu. mm.	average 63, range 13–180
Polymorphonuclear cells (%)	average 7, range 0–25
Mononuclear cells (%)	average 93
Physical characteristics:	
Mucin clot (1:5 dilution)	excellent*
Relative viscosity units	average 235, range 5.7–1,160

* Graded from 0 to 4 as no clot, poor, fair, good, and excellent (Robinson et al.[6]).

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FIG. 1. External view of biopsy needle and hollow inner cutting needle in place. The surgeon's left index finger is on the superior margin of the patella.

Ropes and Bauer has been found convenient. Group I resembles normal synovial fluid and is representative of traumatic, neuropathic and degenerative joint effusions. Group III is representative of septic effusions with marked elevation of the leukocytes, predominance of polymorphonuclear leukocytes and low relative viscosity values. Group II is intermediate, particularly with respect to the cell count. The fluids associated with gouty arthritis and with the inflammatory phase of rheumatic fever are often of this type. Rheumatoid arthritis can be considered representative of this group but may be of group II or group III depending upon the severity of the inflammatory process.

In interpreting the synovial membrane findings one must be aware of the normal variations that occur from one anatomic location in the joint to another. According to Kling,[8] the suprapatellar bursa near the distal part of the quadriceps tendon is lined by a synovialis with sparse cells and few blood vessels overlying the collagenous bundles of the quadriceps tendon. In the proximal portion of the suprapatellar bursa (from which most of the specimens of this study were obtained) the synovialis is well developed, has numerous villi, and in places is lined by cells that may form several rows beneath which there is a synovial tissue of loose collagen fibers, varying amounts of fat and numerous blood vessels.

MATERIAL

This report is based on fifty-five synovial biopsies of the knees of forty-nine patients, two-thirds of whom were in the hospital; the others were outpatients. Synovial fluid was examined in forty-three of these patients. In thirty-five patients the indication for biopsy was to establish the etiology of the joint disease; in the remaining fourteen, in whom the diagnosis was established, the procedure was performed to obtain a clearer concept of the histologic characteristics of the synovium.

The biopsy material was considered satisfactory in all but five patients, or 10 per cent of the group. In these instances the specimens consisted of muscle, inspissated fibrin or tendon, and were obtained from a few patients when the technic was new to us. The single complica-

FIG. 2. Patient M. M., a twenty-seven year old woman. Rheumatoid arthritis of seven years' duration, A. R. A. Stage I;* active process involving knees only. Increased numbers of small blood vessels beneath the synovium; hypertrophy of cells in the walls of the vessels with small lumina. Magnification × 500.

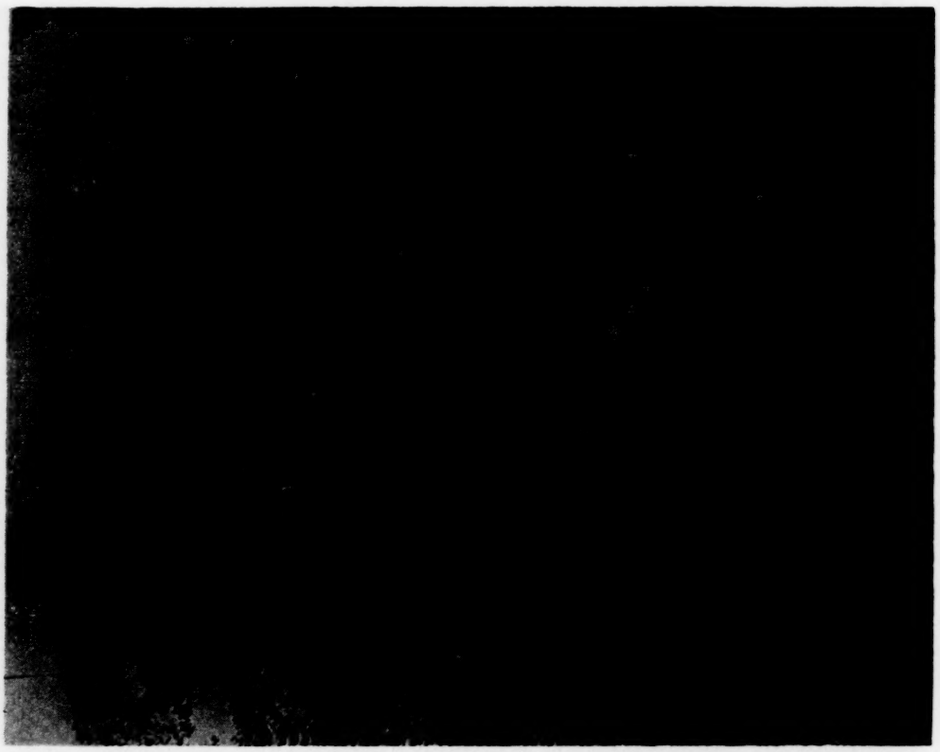

FIG. 3. Patient D. S., a twenty-seven year old woman. Rheumatoid arthritis of three years' duration, A. R. A. Stage I; active process involving one knee only. Increased numbers of blood vessels and inflammatory cellular infiltrations including lymphocytes and plasma cells; occasional areas of apparent proliferation of the lining cells; loss of definition of collagen fibers; subsynovial edema. Magnification × 210.

* Classification of Steinbrocker et al.[9] adopted by the American Rheumatism Association, has been followed in describing the degrees of involvement of the joints in rheumatoid arthritis. Stage I denotes early changes with varying degrees of osteoporosis as the chief radiographic finding; stage IV denotes the most advanced involvement with bony or fibrous ankylosis of joint; stages II and III are intermediate and include some degree of cartilaginous and bony destruction.

FIG. 4. Patient E. K., a forty-three year old man. A rheumatoid process, which had involved multiple peripheral joints four years before, subsided spontaneously without residual joint damage within one year of onset. Six months before this biopsy specimen was obtained of the clinically normal right knee, the right sternoclavicular joint became swollen and painful and on conventional biopsy showed changes similar to these. Chronic rheumatoid arthritis (right knee); thick walled blood vessels beneath synovium; fibrin in joint space. Magnification × 210.

FIG. 5. Patient R. Z. L. W., a fifty-seven year old man. Rheumatoid arthritis of seven years' duration, A. R. A. Stage IV, inactive; heavy inflammatory cellular infiltrations including lymphocytes and plasma cells beneath synovium. Magnification × 210.

tion in the group occurred in a patient with rheumatoid arthritis and consisted of symptomatic hemarthrosis with increased joint pain and swelling for a forty-eight-hour period.

Table II summarizes the clinical data in the twenty-five cases of rheumatoid arthritis. Synovial fluid studies were made in twenty-two patients of this group and in seventeen of these the fluids were of the group II type, with moderately high leukocyte counts, low relative viscosity values, and mucin clot estimated as fair or poor in most instances. In the other five patients the fluid characteristics were of the group I type.

TABLE II

RHEUMATOID ARTHRITIS, CLINICAL SUMMARY, TWENTY-FIVE PATIENTS*

Age at onset	2 to 76 yr. (average 31 yr.)
Duration of disease	3 mo. to 25 yr. (average 6 yr.)
Multiple joint involvement	17 patients
Bi-articular involvement only (knees)	3 patients
Mono-articular involvement only (one knee)	5 patients

* Twelve women, thirteen men.

Synovial membrane specimens in seventeen patients were considered to be characteristic of a rheumatoid process. The most frequently encountered pathologic alterations consisted of: (1) proliferation of the lining cells of the synovium and (frequently) fibroblastic proliferation throughout the thickened synovial tissue; (2) varying degrees of loss of the normally distinct fibrillar appearance and bundle arrangement of the collagen of the synovium; (3) apparently increased numbers of small blood vessels in the subsynovial tissue and increased thickness of blood vessels with hypertrophy of the cells of the vessel walls; (4) inflammatory cellular infiltrations, including lymphocytes and plasma cells, beneath the synovial lining—occasionally the inflammatory cellular infiltration appeared heaviest in the tissues immediately surrounding the blood vessels (in some instances, in both active and relatively inactive rheumatoid processes, the inflammatory cells were seen in dense follicular collections); (5) subsynovial edema that was most apparent in the more active forms of rheumatoid arthritis. (Figs. 2 to 5.)

Five specimens showed some of the features of an inflammatory process but lacked sufficient pathologic changes to suggest more than a mild synovitis without characteristics permitting more precise histopathologic classification. We were unable to correlate such clinical features as duration of disease or stage or activity of the rheumatoid process with the pathologic alterations of the synovium; nor could we establish any conclusive correlation between synovial fluid characteristics and the histologic changes of the synovial membrane. With more experience such correlations might be possible.

REITER'S SYNDROME

Three patients, all men, had a disease characterized clinically as Reiter's syndrome. During the initial course of the illness one of these patients had, in addition to arthritis, conjunctivitis and nonspecific urethritis, an extensive skin lesion of the type of keratodermia blennorrhagica involving the hands and feet. The synovial fluid from two of the patients was of the group II type with a relative viscosity of 4.3 units and 7.5 units, and a leukocyte count of 29,300 and 7,600/cu. mm., respectively. The specimen of the first patient had a differential leukocyte count of eighty-eight polymorphonuclear neutrophils and twelve mononuclear cells per one hundred cells whereas the latter had a differential of nineteen polymorphonuclear cells and eighty-one mononuclear forms. The fluid from the remaining patient had a white blood cell count of 150/cu. mm. Although the patient's condition was in remission and his synovial fluid within nearly normal limits, the histologic features of the synovial specimen were similar to those of the other two patients. The synovial changes in these three specimens were similar to those seen in rheumatoid arthritis but were much less pronounced. Mild reduplication of the synovial lining cells as well as subsynovial edema were present. Inflammatory cellular infiltration was present to only a slight degree. This was in contrast to the clinical severity of joint involvement in the patient from whom the synovium was obtained for Figure 6.

DISSEMINATED LUPUS ERYTHEMATOSUS

Biopsy specimens were obtained in three female patients with well documented clinical evidence of disseminated lupus erythematosus; two had positive "lupus erythematosus" cell preparations at the time of the histologic examination and the other had had positive preparations previously but not at the time of the examination. The two patients with positive "L.E." cell preparations, ages forty-three and sixteen, with disease of nine months' and two

years' duration, respectively, were in acute phases of the disease. The older patient had minimal involvement of the proximal interphalangeal joints in one hand as the only articular manifestations. The joint fluid studies from the asymptomatic knee included a leukocyte count of 50/cu. mm., predominantly mononuclear forms, a relative viscosity of two (possibly falsely low), and a fair mucin clot. The younger patient had an active inflammatory process in most of the peripheral joints and fluid findings of 3,800 leukocytes/cu. mm., 91 per cent mononuclear forms, a relative viscosity of 120 units, and an excellent mucin clot. The other patient, age thirty-three, had had the disease for six years with an intermittently acute course, and exhibited pronounced muscle atrophy and periarticular contractures. At the time of the biopsy she was in a state of remission. Her fluid studies included a leukocyte count of 16,600/cu. mm., predominantly mononuclear cells, a relative viscosity of 6.9 units, and a poor mucin clot.

No distinguishing features were observed in the synovium of these three patients. The changes were minimal and limited to very mild inflammatory cellular infiltration in both the patient in remission and in the patient with minimal joint manifestations. The synovium from the sixteen year old patient with clinically acute inflammatory manifestations showed alterations similar to some of those of the rheumatoid arthritis group. The most prominent features were moderate hyperplasia of the lining cells and mild increase in numbers of blood vessels whose walls were thickened and lumina narrowed.

GOUT

Clinical data for seven patients with gout are tabulated in Table III. Six of these patients were having manifestations of acute gouty arthritis in the biopsied knee joint, three of them in other joints as well. One of these patients had a tophus on the left elbow, the others were without visible tophi. Synovial fluid studies were performed in five of the six patients and in four of these the findings were of the group II type, with a leukocyte count varying between 1,150 and 8,100/cu. mm. Polymorphonuclear cells were predominant in three patients. The relative viscosity values were between 4.4 and 11.2 units, and the mucin clot was graded as fair in all. The joint fluid in one of this group had a leukocyte count of 1,000/cu. mm., all mononuclear forms, an excellent mucin clot, and a normal relative viscosity of 67.8 units. In these six patients with acute symptoms the serum uric acid was elevated in four and normal (3.2 mg. per cent and 4.8 mg. per cent) in the remaining two. Care was taken to insure that no drug which might cause a depression of the serum uric acid had been administered.

TABLE III

GOUT, CLINICAL SUMMARY, SEVEN PATIENTS (MALE)

Age at onset	32 to 80 yr. (average 51 yr.)
Duration from time of first symptoms	2 wk. to 14 yr. (average 5.3 yr.)
Acute gouty arthritis	6 patients
Elevated serum uric acid	4 patients (6.9 to 10.7 mg. %)
Normal serum uric acid	2 patients (4.8 and 3.2 mg. %)
Asymptomatic interval phase of gout	1 patient
Serum uric acid	4.2 mg. %

* Serum uric acid, upper limit of normal in men, 6.0 mg. % (total color method of Block and Geib[10]).

Of interest was the discovery of collections of urate deposits (in specimens preserved in both absolute alcohol and 10 per cent formalin) in the synovial membrane of four of the non-tophaceous patients with acute symptoms. One of the patients, an eighty year old man experiencing his first attack of gout of two weeks' duration, had normal serum uric acid levels (3.2 mg. per cent and 3.1 mg. per cent) while under observation in the hospital. Multiple collections of urate deposits in concentric aggregates, surrounded by a narrow zone of lymphocytes and plasma cells, were seen in the synovial specimens from two of the patients. The serum uric acid in one of these patients was in the normal range and in the other was 10.7 mg. per cent. Urate deposits in very small aggregates, without surrounding inflammatory cellular reaction, were observed in the specimen from a third patient. In a fourth case of non-tophaceous gout urate deposits were present in multinucleated giant cells. (Fig. 7.) This patient was in an asymptomatic interval with a serum uric acid of 4.2 mg. per cent at the time of the biopsy but with a subsequent level of 6.9 mg. per cent. Aside from the finding of urate deposits in these four patients, changes in the synovial specimens in gout were minimal or otherwise not distinctive.

NEUROPATHIC JOINT DISEASE

Biopsy specimens were obtained from three patients with neuropathic joint involvement. Two of the patients had active neurosyphilis and one patient, age fifty, had spina bifida with a flail left lower extremity and on the right (the extremity of the biopsied joint), motor and

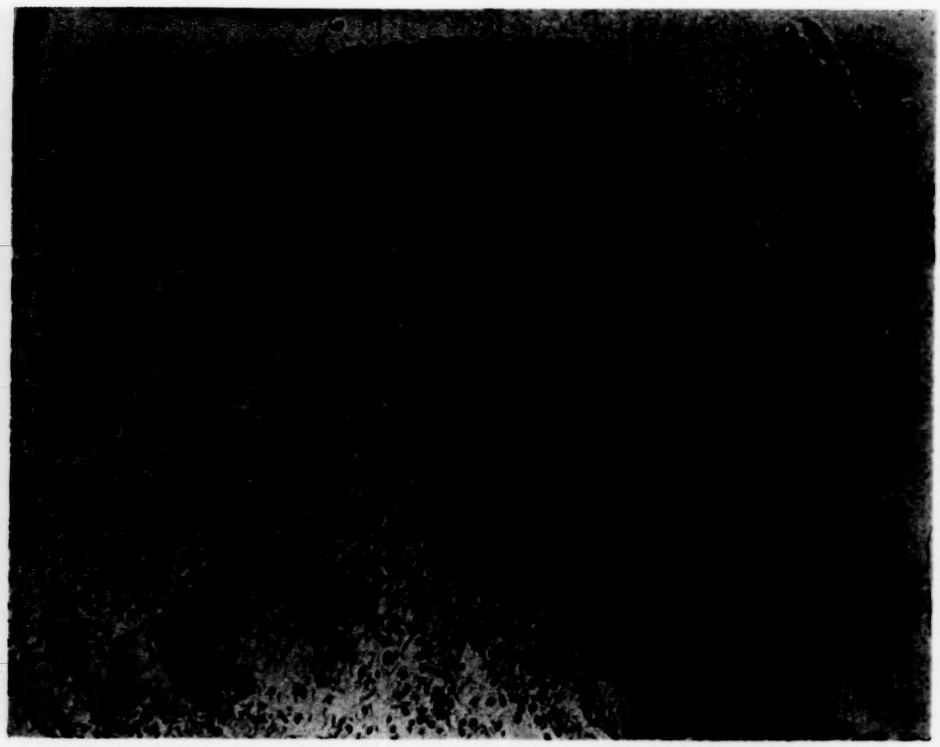

FIG. 6. Patient O. D., a thirty-four year old man. Reiter's syndrome of two months' duration; severe inflammatory process in multiple joints; edema and slight inflammatory cellular infiltration beneath synovium. Magnification × 210.

FIG. 7. Patient M. B., a fifty-nine year old man. Intermittent joint symptoms of fourteen years' duration; gout, non-tophaceous, asymptomatic interval; urate deposits in giant cells beneath synovium. Magnification × 500.

FIG. 8. Patient S. McD., a fifty year old woman. Syphilis of fourteen years' duration; tabes dorsalis; rapidly enlarging right knee with pain of one year's duration; neuropathic joint disease; lime salt deposits beneath synovium. Magnification × 210.

FIG. 9. Patient J. T., a sixty-two year old man. Fever of two weeks' duration, severe right knee pain and swelling of four days' duration; blood and joint fluid culture, coagulase-positive, hemolytic staphylococcus; acute purulent arthritis; purulent exudate in joint space; necrosis of synovial tissue. Magnification × 210.

sensory neurologic deficits with preservation of sufficient function to permit walking while using a brace on the left. In the syphilitic joints the synovial fluid findings were as follows: leukocyte counts of twenty-five and 12,500/cu. mm., differential counts of 100 per cent mononuclear cells, and 73 per cent polymorphonuclear cells and 27 per cent mononuclear cells, relative viscosity values of 2.8 and 10 units, and mucin clot estimations of fair and poor, respectively. The fluid studies in the patient with spina bifida were as follows: leukocyte count of 0 cells/cu. mm., relative viscosity of 12 units, and a fair mucin clot. In both of these patients with syphilis there was a positive blood reaction to the Kahn test and in one of them there was a positive joint fluid reaction.

The microscopic synovial characteristics of these three patients were similar and included an abundance of fibrinoid in the subsynovial tissue and widespread deposits of lime salts in the thickened synovial tissue. (Fig. 8.) Cellular proliferation and infiltration were absent.

OTHER JOINT DISEASES

Among the other diseases in this biopsy series were dermatomyositis, serum sickness arthritis, idiopathic osteoarthropathy, tuberculous arthritis, and one case of acute purulent arthritis caused by a coagulase-positive, hemolytic staphylococcus. Except for the last two diseases, specimens from this group demonstrated no distinctive microscopic features. Several Langhans' type giant cells of an atypical appearance, and considerable fibrous tissue proliferation were the most significant changes in the material from the case of tuberculous arthritis (confirmed by culture). The changes in the synovium of the patient with purulent arthritis consisted of increased vascularity with inflammatory cellular infiltrations, chiefly polymorphonuclear cells, and changes in the architectural pattern of the synovial layers. (Fig. 9.) The initial leukocyte count on the joint fluid of this patient was 389,000 cells/cu. mm., all polymorphonuclear forms.

COMMENTS

The limitations of the punch biopsy are important and must be taken into account when the morphologic features of the synovial specimens obtained by this method are interpreted in relation to the clinical findings. Since the joint surfaces are not seen, the gross findings that direct inspection might yield are not available and the selection of the biopsy sites is one of chance. The matter of chance selection is particularly important in view of the work by Cruickshank[11] in which he was able to demonstrate wide variations in appearance of the synovium from one area of the joint to another in rheumatic diseases. Variations in histologic findings were most striking in instances of rheumatoid arthritis under two years' duration and in stages I and II of acute rheumatoid arthritis. It is in this situation that punch biopsy is most often employed.

In most of the patients in the rheumatoid arthritis group the biopsy was not secured for diagnostic purposes. That the procedure did yield material microscopically characteristic of a rheumatoid process in seventeen of twenty-five patients is of significance. The value of the procedure in differential diagnosis was notable in the cases of mono-articular joint manifestations in which tuberculosis and rheumatoid arthritis frequently are the primary considerations. The similarity in synovial characteristics of the sternoclavicular joint, clinically active, and of the clinically inactive knee in the patient cited in Figure 4 recalls the work of Bick[12] in which he said, "synovial tissue from any one joint bears so close a resemblance to that from other joints when examined microscopically that it cannot be differentiated. This applies both to normal and diseased tissue."

The synovial alterations of rheumatoid arthritis, Reiter's syndrome, disseminated lupus erythematosus and dermatomyositis were qualitatively so similar that the synovial biopsy in this experience was not conclusive in differentiating these diseases. Certain differences, as mentioned previously, were apparent but these were principally quantitative differences that have not proved diagnostically significant.

Finding urate deposits in the synovium of acute gouty joints in non-tophaceous patients in four of six instances was not anticipated. This constituted an absolute diagnosis and settled the diagnostic problem in two patients with normal serum uric acid levels. This situation is frequently encountered and the biopsy procedure offers a prompt source of important information. The absence of urate deposits, of course, would not exclude the diagnosis.

The synovial findings in neuropathic joint diseases were characteristic of these diseases, particularly with respect to the widespread lime salt deposits. The same features may be present

in a joint which is the site of chronic trauma so that these findings are not diagnostic in themselves. In one of the patients, however, the possibility of rheumatoid arthritis had to be considered seriously so that in this instance the biopsy findings were helpful.

The punch biopsy procedure, carried out with a strict aseptic technic, is innocuous and provides a rapid and inexpensive means for obtaining synovial tissue. This information otherwise would have been unavailable in most of the patients in this study. If synovium was to be examined, the alternative would have been conventional arthrotomy and in most instances this was either contraindicated by the patient's illness or considered undesirable because of the expense and period of incapacity involved or the nature of the joint disease. Such a biopsy method cannot be expected to take the place of conventional arthrotomy when the latter procedure is indicated.

SUMMARY

The information obtained by punch biopsy of the synovium of the knee in fifty-five procedures in forty-nine patients with diseases of the joints has been analyzed. One complication occurred in the form of mild, transient, symptomatic hemarthrosis. Diagnoses made on the basis of this procedure included gout, rheumatoid arthritis, neuropathic joint disease and tuberculosis. The procedure is considered an important and practical diagnostic method in joint diseases in those instances in which conventional arthrotomy is inadvisable and other measures have not revealed the diagnosis.

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REFERENCES

1. Forestier, J. Instrumentation pour mèdicale. *Compt. rend. Soc. de biol.*, 110: 186, 1932.
2. Forestier, J. Personal communication.
3. Polley, H. F. and Bickel, W. H. Experiences with an instrument for punch biopsy of synovial membrane. *Proc. Staff Meet., Mayo Clin.*, 26: 273, 1951.
4. Polley, H. F. and Bickel, W. H. Punch biopsy of synovial membrane. *Ann. Rheumat. Dis.*, 10: 277, 1951.
5. Polley, H. F., Bickel, W. H. and Dockerty, M. B. Punch biopsy of synovial membrane in the diagnosis of articular disease. *Ann. Rheumat. Dis.*, 13: 353, 1954.
6. Robinson, W. D., Duff, I. F. and Smith, E. M. Joint fluid changes in rheumatoid arthritis. *J. Michigan State M. Soc.*, 54: 277, 1955.
7. Ropes, M. W. and Bauer, W. Synovial Fluid Changes in Joint Diseases. Cambridge, 1953. Harvard University Press.
8. Kling, D. H. The Synovial Membrane and the Synovial Fluid with Special Reference to Arthritis and Injuries of the Joints Los Angeles, 1938. Medical Press.
9. Steinbrocker, O., Traeger, C. H. and Batterman, R. C. Therapeutic criteria in rheumatoid arthritis. *J. A. M. A.*, 140: 659, 1949.
10. Block, W. D. and Geib, N. C. An enzymatic method for the determination of uric acid in whole blood. *J. Biol. Chem.*, 168: 747, 1947.
11. Cruickshank, B. Interpretation of multiple biopsies of synovial tissue in rheumatic diseases. *Ann. Rheumat. Dis.*, 11: 137, 1952.
12. Bick, E. M. Surgical pathology of synovial tissue. *J. Bone & Joint Surg.*, 12: 33, 1930.

A Clinical, Physiologic and Biochemical Study of Patients with Malignant Carcinoid (Argentaffinoma)*

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RECENTLY, several groups have described an unusual syndrome associated with malignant carcinoid of the small intestine with metastases to the liver.[1–7] This syndrome is manifested principally by flushes, "cyanosis," chronic diarrhea, respiratory distress and valvular disease of the heart. Although these protean manifestations are in some way related to carcinoid tumors, suggestions concerning a mechanism by which these changes are produced are speculative.

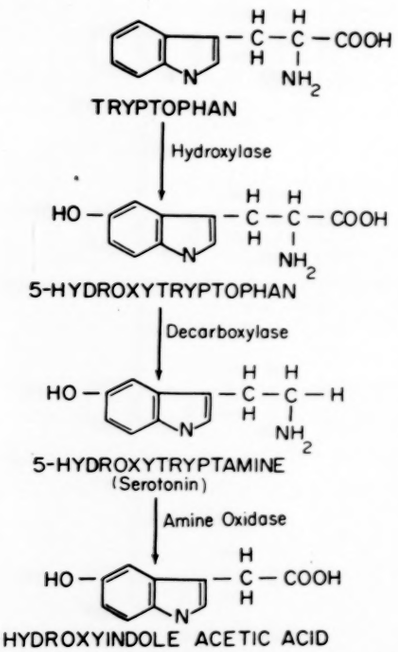

FIG. 1. The 5-hydroxyindole pathway of tryptophan metabolism.

Thorson et al.[2] were the first to suggest that the syndrome might result from secretion of 5-hydroxytryptamine (serotonin) by the tumor. This substance was first isolated and identified by Rapport et al.[8,9] as a vasoconstrictor material released by blood platelets during clotting.[10] In earlier studies, Vialli and Erspamer[11,12] had isolated from intestinal mucosa a substance which they termed "enteramine;" later they showed it to be identical with serotonin.[13] Erspamer has suggested that the chromaffin cells of the gastrointestinal tract are in effect an endocrine organ secreting serotonin. Since carcinoid tumors are thought to be derived from the enterochromaffin or argentaffin cells, such tumors might be rich in serotonin. The presence of large amounts of serotonin (1 to 3 mg. per gm.) in carcinoid tumors was actually demonstrated by Lembeck.[14,15] The finding in carcinoid tumors of an agent which has a potent action on smooth muscle in blood vessels, bronchi and intestine[16,17] was the reason for Thorson's implication of serotonin in this syndrome.

Studies on experimental animals showing serotonin to be derived from the amino acid tryptophan[18] suggested that there might also be an abnormality of tryptophan metabolism in this condition. The steps in this 5-hydroxyindole pathway of tryptophan metabolism are shown in Figure 1. The end product, 5-hydroxyindoleacetic acid (5-HIAA), is formed from serotonin by oxidative deamination[19] and is excreted in the urine. The urinary excretion of 5-HIAA is an index of this route of metabolism. Initial observations[20] indicated that the excretion of

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5-HIAA by patients with the "carcinoid syndrome" was of such magnitude as to require major utilization of dietary tryptophan for the formation of 5-hydroxyindole compounds.

This report presents the results of an extensive investigation of tryptophan and 5-hydroxyindole metabolism in several patients with malignant carcinoid. These studies support the postulate that excess serotonin production is indeed involved in the "carcinoid syndrome." However, serotonin production is so great that it results in a profound disturbance of tryptophan metabolism. Both of these factors must be considered in attempting to understand the mechanisms of the pathology involved.

MATERIALS AND METHODS

Four patients with the "carcinoid syndrome" were studied by us. Urine samples were made available in a fifth patient, and urine samples plus a specimen of carcinoid tumor tissue were obtained in a sixth patient.

Serotonin* in blood, urine and tissues was measured by the methods of Udenfriend, Weissbach and Clark.[21] Urinary 5-HIAA† was measured by the method of Udenfriend, Titus and Weissbach.[22] Details of the isotopic and paper chromatographic procedures on urine and tissue extracts are presented elsewhere.[23] The analyses involved in the execution of the nitrogen balance study were essentially as described by Reifenstein, Albright and Wells.[24] A special formula diet was prepared in the manner described by Ahrens et al.[25]

CASE REPORTS

CASE 1. A thirty-eight year old white woman was studied for two weeks in December 1954 and again in January 1955. The illness began in 1928 at age eleven when she began to have episodic flushes. Surgical drainage was required for the treatment of a right empyema at age seventeen, and at this time the liver was found to be enlarged. A cardiac murmur was discovered in 1944. Blue discoloration of the face, previously intermittent, became persistent in 1946. Menses ceased in 1947. Fluid accumulation in the legs, abdomen and chest occurred in 1948 and was subsequently controlled by diuretic measures. Diarrhea was not a prominent symptom but had been present intermittently for several years, and since 1948 a gradual weight loss of 25 pounds had occurred. In 1949 she apparently had had a low grade fever for several weeks, the cause of which could not be determined. The patient had been studied at several hospitals since 1948 and on the basis of clinical and cardiac catheterization observations was considered to have pulmonic stenosis and tricuspid insufficiency secondary to rheumatic or congenital heart disease. Peripheral arterial oxygen saturations in 1949 and 1952 were reported to be 91 and 87 per cent, respectively, and the possibility of a small right-to-left shunt through a patent foramen ovale was considered. A long history of emotional difficulties was elicited, and on one occasion she was considered to be frankly psychotic.

On admission the patient's weight was 50 kg., blood pressure 120/80, pulse rate 100 per minute, respirations 20 per minute and oral temperature 36.4°c. She appeared chronically ill and extreme emotional lability was exhibited. The skin of the face appeared cyanotic and there was extensive telangiectasia of the legs below the knees. Frequent flushes were observed, consisting of intense erythema of the face and upper chest with varying involvement of other areas by blotchy, purplish-red patches, the episodes terminating in a briefer phase of blanching. During flushes she felt "sick all over" and complained of severe weakness. The trachea deviated to the right and the superficial neck veins were distended in the upright position. The deep and superficial neck veins exhibited systolic expansile pulsations. The right chest was less prominent than the left, relatively immobile; dullness on percussion and diminished breath sounds by auscultation were noted below the right third rib posteriorly. A diffuse precordial thrust during systole was noted and the left border of cardiac dullness was at the anterior axillary line in the sixth left intercostal space. A systolic thrill was palpable in the third left intercostal space near the sternum and a grade III systolic murmur was audible in this area which lessened in intensity elsewhere over the precordium; the heart sounds were otherwise normal. The abdomen was distended and a fluid wave was demonstrable. The spleen was palpable and the liver was enlarged to 8 cm. below the right costal margin. A single 3 cm. nodule was felt on the surface of the liver. Aside from slight pitting edema of both ankles no other physical findings of significance were noted.

Routine blood counts, platelet counts, blood serologic examination and urinalysis gave normal results. An electrocardiogram was interpreted as consistent with right ventricular hypertrophy and strain. Chest x-ray examination showed pleural thickening and decreased aeration on the right with a right tracheal shift and marked elevation of the right leaf of the diaphragm. Minimal enlargement of the heart was present with slight shift to the left. During the patient's hospitalization at the Walter Reed Army Hospital in August 1954, fasting blood sugar, blood urea nitrogen, serum electrolytes and liver function studies gave results within normal limits. Lateral chest x-ray

* Serotonin was supplied as the creatinine sulfate salt by Dr. Merrill Speeter of the Upjohn Company and by Dr. Kenneth Hamlin of Abbott Laboratories.

† Five-HIAA was also made available by Dr. Speeter of the Upjohn Company. Two-C^{14}-*DL*-tryptophan was obtained from Tracerlab, Inc.

showed fullness of the retrosternal space consistent with enlargement of the right portion of the heart, and barium studies of the entire gastrointestinal tract showed, as the sole abnormality, a 3 by 4.5 cm. diverticulum of the second portion of the duodenum with a possible filling defect within the diverticulum.

The condition was unchanged during the two brief hospitalizations. Permission for liver biopsy examination or abdominal exploration was refused.

This patient was seen again on July 21, 1955, complaining of low back pain and pain and stiffness of the fingers of six weeks' duration. Examination revealed tenderness of the second and third proximal interphalangeal joints and the first and second metacarpophalangeal joints bilaterally. The erythrocyte sedimentation rate (Westergren) was 35 mm. per hour and the sheep cell agglutination test for rheumatoid arthritis was negative (titer of 4). A lupus erythematosus cell preparation gave negative results. Total serum proteins were 6.8 gm. per cent and the only electrophoretic abnormality was a diminished albumin of 3.4 gm. Roentgenograms of the hands and lumbar spine showed no bony abnormalities.

CASE II. A fifty year old white woman was studied from March 3, 1955 to July 28, 1955. Episodes of flushing were first experienced in 1946 and were ascribed to the menopause. Estrogens gave no relief. She was examined in 1948 because of cramping abdominal pains and the liver was found to be enlarged. She felt well thereafter until September 1952 when the flushes became severe and were accompanied by anorexia, vomiting, weakness and a rapid weight loss of 15 pounds. During hospitalization in November 1952, the liver edge was found to be about 18 cm. below the right costal margin. Roentgen examinations of the gallbladder, kidneys and entire gastrointestinal tract revealed no intrinsic lesions. Liver function tests gave entirely normal results. The patient improved with symptomatic therapy and blood transfusions. An exploratory laparotomy in November 1952 revealed numerous tumor nodules in the liver which upon biopsy examination proved to be carcinoid. The site of the primary tumor could not be determined. She continued to have flushes each day but was otherwise well until September 1954 at which time a recurrence of severe flushing occurred with associated periodic vomiting and diarrhea. She lost an additional ten pounds and for one week prior to admission was incapacitated by these symptoms.

On admission the patient's weight was 37.0 kg., blood pressure 130/80, pulse rate 100 per minute, respirations 20 per minute and oral temperature 37.0°C. She was emaciated, appeared chronically ill and exhibited frequent flushes of two to ten minutes' duration. The flushes began as an intense erythema of the face, upper trunk and palms, were frequently accompanied by blueness of the lips and purplish-red patches on the trunk and extremities, and ended with a brief period of intense blanching. During severe flushes there was swelling of the face and fingers, excessive perspiration and pilomotor activity, subjective sensations of warmth and tingling of the face, nausea and an urge to void or defecate. Scattered senile angiomas were on the skin of the trunk. A faint systolic murmur was audible at the cardiac base but the heart sounds were otherwise normal and no cardiomegaly was apparent. Other positive physical findings were confined to the abdomen which was protuberant due to an tremendously enlarged, nodular liver, the edge of which extended to the pubis and the iliac crests.

Except for mild normocytic anemia, routine blood counts revealed no abnormalities. Total serum proteins were 7.8 gm. per cent and the only abnormality of the electrophoretic pattern was a somewhat diminished albumin of 3.6 gm. Roentgen examination of the hands at the time of arthritic symptoms showed generalized osteoporosis with normal joint spaces. A sheep cell agglutination test for rheumatoid arthritis at this time gave positive results in a titer of 64, and the erythrocyte sedimentation rate (Westergren) was 34 mm. per hour. The following tests and procedures gave normal results or were negative: bleeding time, clotting time, prothrombin time, clot retraction, capillary fragility, platelet counts, lupus erythematosus cell preparation, blood urea nitrogen, fasting blood sugar, serum electrolytes, tests of liver function, urine 17-OH-corticosteroids, chest x-ray, electrocardiogram, right heart catheterization and arterial oxygen saturation.

The patient improved rapidly with symptomatic therapy, although she continued to have several flushes each day. Her appetite improved and she gradually gained 10 kg. In March a low grade fever developed on two occasions, with oral temperature to 38.4°C. The cause of fever could not be determined but on both occasions it subsided within four days. The hospitalization course was also complicated by the gradual development of pain, swelling and stiffness of the fingers with tightening of the flexor tendons and bilaterial flexion contractures of the fifth finger. Examination revealed swelling and tenderness of the interphalangeal joints bilaterally. The physical findings plus a positive reaction to the sheep cell agglutination test were thought to be consistent with rheumatoid arthritis. Satisfactory improvement followed the use of salicylates, local heat and exercise. On discharge from the hospital she was able to return to her duties as a housewife.

CASE III. A fifty-five year old white man was studied from April 7, 1955 to June 21, 1955. He complained chiefly of intermittent, explosive diarrhea of two years' duration accompanied by increasing fatigue and a weight loss of ten pounds. He had been well prior to 1953, although enlargement of the liver was known to have existed since 1940. Erythematous flushes of the face and neck had been present daily since July 1954. Needle biopsy of the liver in November 1954 was thought to show "hepatoma." However,

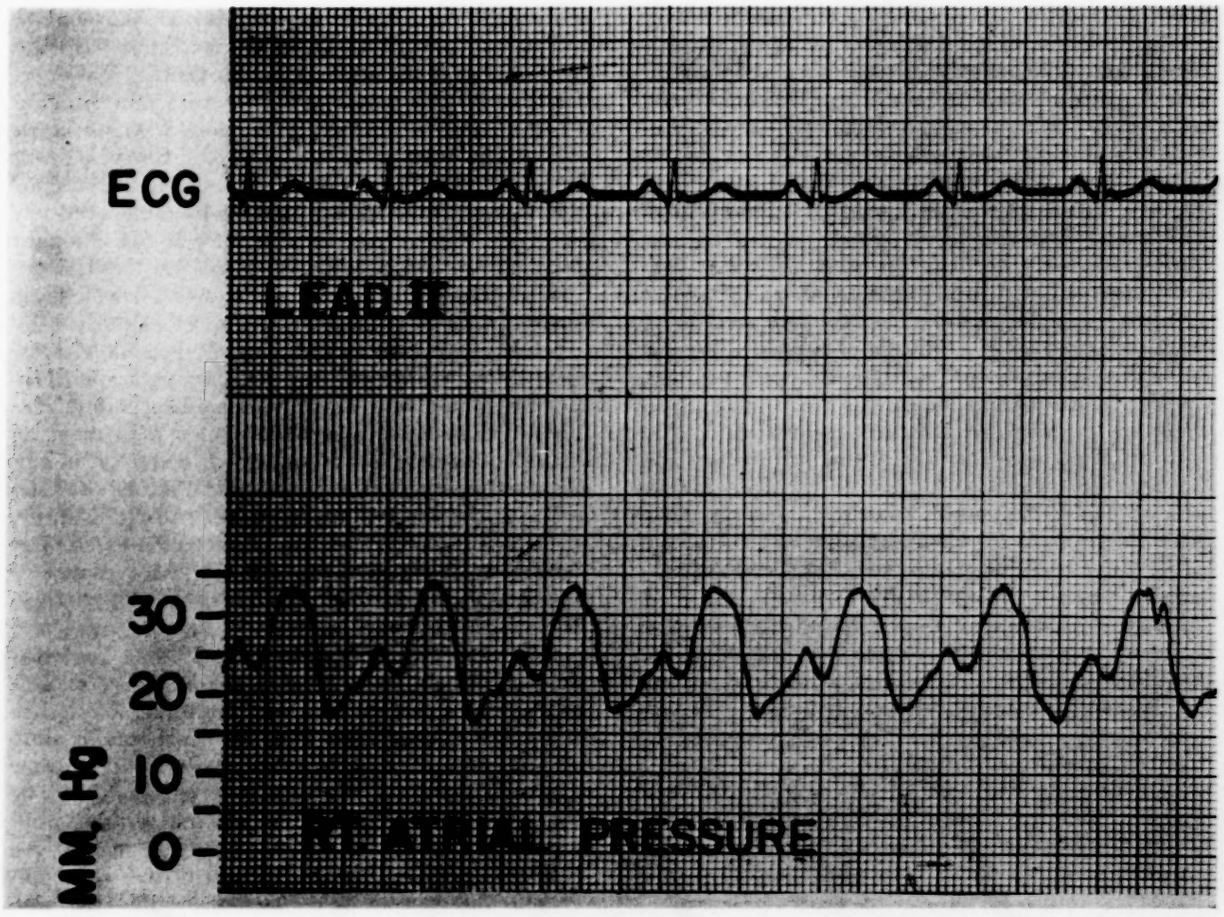

FIG. 2. Right atrial pressure of the patient in Case III showing a large systolic wave characteristic of tricuspid insufficiency.

on exploration the following month two small tumors were excised from the ileum and extensive metastatic tumor was seen in the liver. Diagnosis of the pathology of the primary and metastic lesions was carcinoid. The flushes and diarrhea were unabated and during 1955 dependent ankle edema and paroxysmal respiratory distress characterized by slightly productive cough, dyspnea, substernal oppression and rhinorrhea also developed. Other past illnesses included pulmonary tuberculosis treated with pneumothorax from 1923 to 1926 with subsequent incomplete re-expansion of the right lung. He was hospitalized for one week in 1949 because of fever of undetermined origin and at this time intravenous pyelography revealed a non-functioning right kidney thought to be related to traumatic injury to that kidney which occurred in 1916.

On admission the patients' weight was 77.1 kg., blood pressure 115/80, pulse rate 82 per minute, respirations 20 per minute and oral temperature 36.4°C. He appeared well on examination but exhibited frequent crimson flushes of the face and neck with variable dispersal of blotchy erythema to the trunk and extremities. He was usually unaware of the flushes but occasionally experienced a sense of warmth and tingling of the face. The flushes persisted for one to three minutes and were superseded by a briefer period of cutaneous blanching. Scattered senile angiomas appeared on the skin of the trunk. Systolic expansile pulsations of the deep jugular veins were observed. The left hemithorax was more prominent than the right and relative dullness was noted on percussion as were diminished breath sounds by auscultation over the entire right hemithorax. Wheezes were audible over both lung fields during paroxysms of cough and dyspnea. The heart apparently was not enlarged and the heart sounds were normal. The upper abdomen was protuberant due to enlargement of the liver to the umbilicus. Several nodules of varying size were palpable on the surface of the liver. Vasomotor episodes were induced repeatedly by manual massage over the liver. The splenic tip was palpable. Two plus pitting edema of both ankle and pretibial regions was noted. The physical examination revealed no other abnormalities.

Routine blood counts, blood serologic tests and urinalysis gave normal results. A sulfobromophthalein test showed 23 per cent dye retention in forty-five minutes. Other liver function tests gave normal re-

sults. Total serum protein was 7.6 gm. per cent with 3.6 gm. of albumin and 4.0 gm. of globulin. The following tests and procedures gave normal results or were negative: blood urea nitrogen, fasting blood sugar, total serum cholesterol, platelet counts, prothrombin time, erythrocyte sedimentation rate, bleeding and coagulation times, clot retraction, capillary fragility, serum electrolytes, urinary 17-OH-corticosteroids and feces for occult blood. Several electrocardiograms were normal. On chest x-ray examination extensive pleural and parenchymal changes were noted on the right, and the frontal plane area of the heart measured 25 per cent above normal. X-ray of the lumbar spine showed mild hypertrophic changes. Cardiac fluoroscopy at the time of right heart catheterization revealed an enlarged right atrium. The right atrial pressure was 34/17 mm. of mercury and the pressure curve was typical of tricuspid insufficiency. (Fig. 2.) Tricuspid stenosis was also suspected but unfortunately the catheter could not be passed through the tricuspid valve. Peripheral arterial oxygen saturation was 95 per cent.

A rapid weight loss of 4 kg. followed the restriction of dietary sodium to 800 mg. per day and thereafter the patient's weight was constant. Attacks of respiratory distress were controlled by inhalations of isopropylarterenol aerosol (1:200). Paregoric controlled the diarrhea effectively. Severe low back pain was a prominent early symptom which responded to local heat and exercises. He continued to have from two to twenty flushes daily without associated discomfort. He felt well at the time of discharge from the hospital and planned to return to his work as a sales executive.

CASE IV. A forty-one year old white man was studied from June 19, 1955 to the present. He had experienced four attacks of severe flushing during the previous year and was admitted during a fifth such episode. He felt well in August 1954 when he was first seized by an attack of sudden, intense, erythematous flushing of the face and neck regions accompanied by periorbital and facial edema, palpitations, wheezing and dyspnea. These symptoms subsided within twenty-four hours as did a similar episode in November 1954. The third attack occurred in December 1954 and was accompanied by intractable retching with vomiting and profuse watery diarrhea. This attack subsided after two weeks of hospitalization. At that time an enlarged, nodular liver was discovered and there was transient azotemia. A liver biopsy specimen obtained on December 18 was interpreted as showing "hepatoma" but in retrospect the sections were considered to represent carcinoid. The patient was unable to return to work thereafter because of persisting weakness and in March 1955 suffered another attack similar to that in December and this was successfully managed at home with bed rest and sedation. Since March transitory facial erythema was observed on at least three occasions. He was admitted for study on the third day of another violent attack. A gradual weight loss of thirty pounds had occurred during the previous year and he complained of unrelenting anorexia. Since 1946 the only other significant illness was "arthritis." The onset was gradual with development of severe pain, swelling and inflammation involving the elbows, wrists, knees and proximal portion of the right foot. Severe pain in the hip and lumbar regions also occurred. He was unable to work for eighteen months but improved following a course of gold therapy for what was thought to be rheumatoid arthritis. Since 1947 he has had occasional pain in the shoulders and low back which was easily controlled with salicylates and local heat.

On admission his weight was 76.0 kg., blood pressure 90/50, pulse rate 150 per minute, respirations 40 per minute and rectal temperature 38.6°C. Examination revealed a well developed man in acute distress who sat up frequently to retch or vomit. Diaphoresis occurred frequently. The skin of the face, neck, upper chest and shoulders showed a fiery, violaceous-red color and was warm to the touch. The periorbital and facial areas were markedly edematous and there was marked congestion of the conjunctivae The visible mucous membranes of the nose and mouth were also fiery red. Several senile angiomas were present on the anterior chest. Scattered expiratory wheezes were audible over both lung fields. The heart was not enlarged and the heart sounds were normal; however, a grade III apical systolic murmur was audible during the period of tachycardia. The abdomen was generally distended and shifting dullness to percussion was noted in the flank regions. An enlarged, nodular liver filled most of the upper abdomen, the edge being 17 cm. below the right and 8 cm. below the left costal margin in the mid-clavicular lines. A 2 by 2 cm. bony prominence was noted on the dorsum of the right foot but there was no limitation of joint motion. No other significant findings were observed.

On admission routine blood counts were normal except for a white blood cell count of 19,200 per cubic mm. and a differential count of 82 neutrophils, 10 lymphocytes and 8 monocytes. Routine urinalysis showed 10 to 15 erythrocytes per high power field in the sediment but otherwise revealed no abnormalities. Chemical analyses of whole blood and serum gave the following results: total protein 6.3 gm. per cent, with albumin 2.9 gm. and globulin 3.4 gm.; urea nitrogen 90 mg. per cent, creatinine 3.8 mg. per cent; uric acid 16.4 mg. per cent; fasting sugar 119 mg. per cent; total cholesterol 128 mg. per cent; sodium 121 mEq./L; potassium 4.5 mEq./L; CO_2 content 16 mEq./L; chloride 85 mEq./L; calcium 9.6 mg. per cent, and phosphorus 9.6 mg. per cent. With the exception of a persisting low serum albumin (confirmed by electrophoretic studies) and uric acid averaging 8.0 mg. per cent, repetition of these tests two weeks after admission revealed uniformly normal values. A sulfobromophthalein test showed 9 per cent dye retention in forty-five minutes but the results of

other tests of liver function were within normal limits. The erythrocyte sedimentation (Westergren) rate was 25 mm. per hour. The following tests or procedures were also carried out at least two weeks after admission and gave normal results: urea and phenol red clearances, platelet counts, clot retraction, bleeding time, prothrombin time, clotting time, lupus erythematosus cell preparation, capillary fragility, sheep cell agglutination for rheumatoid arthritis, arterial oxygen saturation, right heart catheterization and feces for occult blood. Chest x-rays were interpreted as showing a small encapsulated pleural effusion in the right horizontal fissure, a poorly defined density at the right heart border and two small circumscribed densities at the right posterior lung base consistent with metastatic tumor. Roentgen survey of the entire bony skeleton showed the only abnormalities to be increased bone density, areas of cystic degeneration and presumed destruction of articular cartilage in the right proximal foot and right wrist areas. Although gout was suspected, the observations were thought most closely to approximate those seen in rheumatoid arthritis. Intravenous pyelography revealed changes suggestive of a tumor or cyst at the superior pole of the right kidney. Complete roentgen examination of the gastrointestinal tract performed elsewhere in June 1955 showed no intrinsic lesions of the bowel. Serial electrocardiograms revealed no abnormalities except for tachycardia initially.

The acute episode of this patient disappeared within a week after admission through judicious use of sedatives, antispasmodics and forcing of fluids. During the first two weeks a weight loss of 8 kg. occurred which probably represented a diuresis of ascitic fluid. Weight gain has been gradual since that time with excellent food intake. Further studies are in progress.

Case v. A young adult man died on October 29, 1954 at the University of California Hospital, San Francisco, California, after an illness of about two and one-half years which was characterized by striking reddish-blue discoloration of the skin, diarrhea, dyspnea and right heart failure. In June 1954 his blood pressure was 130/80 and systolic pulsations of the neck veins were noted. He failed to recover following an abdominal operation on October 19, 1954. Postmortem examination revealed a small carcinoid tumor in the ileum with extensive metastases to the liver and peritoneal nodes, pulmonic stenosis and right ventricular hypertrophy.

Case vi. A fifty-two year old woman was admitted to the Mayo Clinic in May 1955, following discovery of a pelvic mass by her local physician. She complained of persistent watery diarrhea since an operation in 1950 and frequent flushes since 1953. A cholecystectomy in 1950 revealed a mass in the ileocecal region. This was resected and the diagnosis of carcinoid tumor made by the pathologist. On examination vasomotor episodes could be produced by manipulation of the remaining tumor. Exploration of the abdomen was carried out on June 9, 1955, and multiple omental and peritoneal metastases were found. The liver appeared normal. A 38 gm. tumor was resected. During manipulation of the abdominal tumors a fall in blood pressure was noted. Postoperatively the patient was given x-ray radiation and no flushing episodes occurred during fifteen days of observation.

COMMENTS ON CASE REPORTS

In all but one of the six patients histologic proof of extensive carcinoid tumors was seen. The opportunity to observe four of these patients during periods of prolonged hospitalization made it possible to evaluate previous observations which were based largely on isolated observations and autopsy records. Although the clinical manifestations reported by Thorson et al.[2] were observed, all were not present in each patient. Cutaneous vasomotor episodes of varying severity and chronic diarrhea were present consistently. A cyanotic appearance was discernible only in the patients in Cases i and v. The "cyanosis" occurred in the absence of significant arterial anoxia and probably resulted from physiologic and anatomic changes in the cutaneous vascular bed, as described previously.[2] Respiratory distress with a bronchospastic component was present in half the patients (Cases ii, iv, v) as was the development of pulmonic stenosis or tricuspid insufficiency (Cases i, iii, v). Cardiac involvement seems to occur late in the course of this disorder. The patient in Case vi, to our knowledge, is the only reported instance of certain features of the syndrome (diarrhea and flushes) in the absence of demonstrable liver metastases. An additional finding was the development of hypotension during flushes, this being most marked in the patients in Cases ii (vide seq.) and iv. The presence of arthritic symptoms in our four patients suggests the possibility of a more generalized connective tissue disorder than that affecting the heart valves and endocardium. The azotemia during flushing episodes in the patient in Case iv appeared to result from prerenal factors. The profound diuresis which followed cessation of the episode suggested the operation of an antidiuretic mechanism during the flush. These observations are consistent with the actions of serotonin on the kidney as reported by Erspamer.[16]

EXPERIMENTAL TESTS

Five-Hydroxyindole Compounds in Blood, Cerebrospinal Fluid, Urine and Feces. As shown in Table

I, blood levels of serotonin in carcinoid patients were greatly elevated above normal values. All the circulating serotonin was found in the platelets, none could be detected in plasma (<0.02 μg. per ml.). No significant difference occurred in platelet counts or serotonin levels in simultaneous blood samples from the antecubital vein, right atrium, pulmonary artery and femoral artery (Cases II, III, IV). Serotonin could not be detected in cerebrospinal fluid (Cases II and IV). Urinary excretion of serotonin in normal subjects was less than 0.1 mg. per 24 hours whereas in carcinoid patients it ranged from 1 to 2 mg. per day.

TABLE I
FIVE-HYDROXYINDOLE COMPOUNDS IN BLOOD AND URINE

Subjects	Blood Serotonin (μg./ml.)	Urine 5-HIAA (mg./24 hours)	Total Urine 5-OH-Indoles (mg./24 hours)
Normal	0.1–0.3*	2–9*	.....†
Carcinoid:			
I	2.5	320–392	453
II	0.5–1.5	240–280	345–385
III	1.2–1.9	380–580	460–865
IV	1.7–2.7	214–572	336–640
V	.....	140	.....
VI	.....	76	.....

* The range of values in about 40 non-carcinoid persons.

† Measurement not feasible on normal urine because of large dilution required to eliminate interfering substances.

Previous studies from this laboratory[22] have shown that 5-hydroxyindoleacetic acid (5-HIAA) is a constituent of normal urine. The excretion of this metabolite of serotonin in the urine of all the carcinoid patients was many times that of normal. (Table I.) No 5-HIAA could be detected in plasma or cerebrospinal fluid (<0.5 μg. per ml.), and none was found in feces.

Five-hydroxyindole compounds other than serotonin and 5-HIAA were found in appreciable quantities in carcinoid urine. Direct application of the nitroso-naphthol color test to urine gave values for total 5-hydroxyindoles 10 to 50 per cent higher than values obtained with the specific extraction procedure for 5-HIAA. (Table I.) The unidentified fraction was found to consist largely of two substances (X and Y) which were detected by their solubility characteristics and their chromatographic behavior. (Table II.) No 5-hydroxytryptophan could be detected and no trace of N-methylated serotonin analogs was found.[26]

Studies on Carcinoid Tumor. A 38 gm. carcinoid tumor was removed from the patient in Case VI at the Mayo Clinic and a 22 gm. portion of this neoplasm was frozen immediately and sent to this laboratory for chemical and enzymatic studies. The total 5-hydroxyindole content of the tumor was 1.1 mg. per gm. of which 0.8 mg. per gm. was serotonin and only a trace was 5-HIAA. The two unidentified 5-hydroxyindoles present in urine were also detected in extracts of the tumor. Although homogenates and minces of the tumor did not form 5-hydroxyindole compounds from tryptophan,* catalysts were demonstrated for two steps of the 5-hydroxyindole metabolic pathway, 5-hydroxytryptophan decarboxylase[27] and monoamine oxidase.[19]

TABLE II*
CHROMATOGRAPHIC BEHAVIOR OF FIVE-HYDROXYINDOLE COMPOUNDS

Compound	R_f	Occurrence in Carcinoid Urine
X	0.02	+
5-Hydroxytryptophan	0.10	–
5-Hydroxyindoleacetic acid	0.20	+
5-Hydroxytryptamine	0.60	+
Y	0.70	+
N-Dimethyl serotonin	0.90	–

* Chromatograms developed with n-propanol—1N NH_3 (5:1) and sprayed with nitroso-naphthol reagent.

After removal of the tumor, urinary excretion of 5-HIAA by the patient fell from a level of about 76 to 44 mg. per 24 hours. The persistence of an elevated urinary 5-HIAA agreed with the surgeon's report that numerous metastases were not resectable. Any quantitative interpretation of the drop in urinary 5-HIAA after surgery, for example calculation of the percentage of tumor mass remaining, may be misleading since no effort was made to maintain a constant tryptophan intake during the periods of analysis.

Relationship of Dietary Tryptophan to Production of 5-Hydroxyindole Compounds. Unless one sup-

* The tryptophan hydroxylating enzyme is not readily demonstrable in any isolated mammalian tissue. Therefore failure to find it in the tumor does not mean that this tissue is incapable of carrying out this reaction *in vivo*.

poses a *de novo* synthesis of the indole ring, one must consider tryptophan to be the dietary precursor of 5-hydroxyindoles. The only direct evidence for the conversion of tryptophan to these substances comes from studies on bacteria,[28] toads and rabbits.[23]

In man, in whom 5-hydroxyindoles normally represent only a small fraction of the indole compounds of the body, one would expect the amount of tryptophan normally ingested to saturate the 5-hydroxyindole-forming catalysts adequately. Thus in preliminary studies on human volunteers the urinary excretion of 5-hydroxyindoles was relatively constant over wide ranges of tryptophan intake. In dogs also administration of 5 to 10 gm. of tryptophan causes only a slight rise in the urinary 5-HIAA.[23]

In marked contrast to the situation in normal human subjects, the excretion of these substances by carcinoid patients varies with the amount of tryptophan in the diet. The following experiment demonstrated this relationship. One of the patients (Case III) was fed a constant diet* designed to give a daily tryptophan intake of 500 mg. The daily urinary excretion of total 5-hydroxyindoles and that present as 5-HIAA were measured and the nitrogen balance was determined. At stated intervals the tryptophan intake was increased by the addition of crystalline *l*-tryptophan to the diet.

It is obvious from the results noted in Figure 3 that at a daily intake of 500 mg. (2.45 mM) as much as 60 per cent of the dietary tryptophan was converted to 5-hydroxyindoles. In spite of this the patient gave no indication of negative nitrogen balance and his weight remained constant. Less than 200 mg. of tryptophan was adequate to fulfill minimal daily requirements of this amino acid. This value is consistent with the report by Rose et al.[30] on the tryptophan requirement of normal man. It is likely that more 5-hydroxyindole compounds are formed than are excreted in the urine since in recent studies we have shown that serotonin administered to normal subjects is recovered to the extent of only about 80 per cent as 5-HIAA in the urine. When the amount of tryptophan was increased to 1400 mg. per day there was a marked rise in the urinary excretion of 5-hydroxyindole compounds which persisted for the duration of the increased intake and returned to the previous levels when the original intake was resumed. A loading experiment with 3500 mg. of tryptophan resulted in an even greater output of these substances. The absolute increase was not proportional to the tryptophan intake, suggesting that the 5-hydroxyindole-forming capacity was being reached. These experiments establish tryptophan as the dietary precursor of serotonin and its metabolites in man. Confirmatory evidence was obtained from experiments using isotopically labelled tryptophan. Twenty microcuries of 2-C^{14}-*dl*-tryptophan was given orally to patients in Cases I, II and III and urinary 5-HIAA was isolated and counted in a GM counter. In each case the isolated 5-HIAA was found to be radioactive, indicating its derivation from the administered tryptophan.

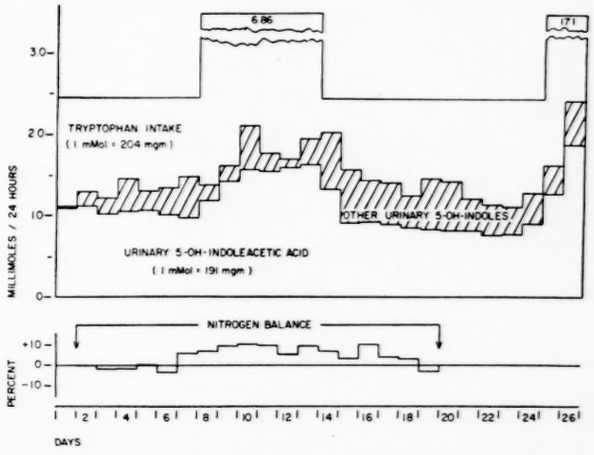

FIG. 3. The effect of variations in tryptophan intake on nitrogen balance and urinary excretion of 5-hydroxyindole compounds.

Studies Relating to Vasomotor Disturbance. Each of the patients exhibited periodic flushing which varied in duration and severity. An accompanying feature was a diminution in the blood pressure (Fig. 4) and constriction of the superficial cutaneous veins, all of which could be duplicated by infusion of serotonin into normal subjects. Emotional disturbances, physical exertion and, in two patients, manipulation of the tumor were the most consistent factors in precipitation of flushes. Although it seemed likely that this manifestation of the syndrome was in some way related to an increase in circulating serotonin, it

* The diet was prepared by homogenizing the following ingredients: lonolac powder, gelatin, dextrose, corn oil, sodium chloride, multivitamins, methionine and water. The patient ingested each day a weighed portion containing 65 gm. protein, 82 gm. fat, 344 gm. carbohydrate, adequate vitamins and essential amino acids in an amount recommended by Rose.[29]

was not possible to demonstrate a significant change in blood serotonin accompanying flushing. This observation is at variance with that of Pernow and Waldenström[31] and of Langemann[32] who reported marked elevations in blood serotonin of carcinoid patients during a flush. The

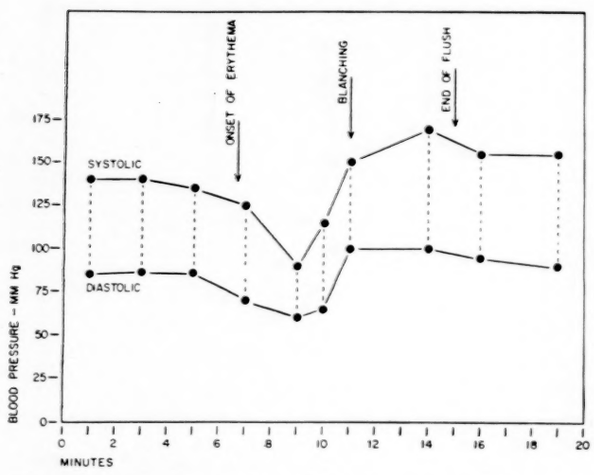

FIG. 4. Blood pressure alterations of the patient in Case II during a "flush."

blood levels reported by these investigators were obtained by bioassay procedures which may not be as specific as the chemical method used in these studies. It is possible that a vasoactive substance other than serotonin is released by the tumor. However, although no rise in circulating serotonin could be established, it is the author's opinion that this agent is responsible for the vasomotor phenomena. Studies in this laboratory[33] indicate that the bulk of circulating serotonin is firmly bound in platelets. This bound form of serotonin, markedly elevated in carcinoid patients, presumably is not responsible for the flushes. Unbound serotonin, if it exists, must be present in plasma in amounts less than 0.02 μg. per ml. It is likely that variations of free circulating serotonin at this level can account for the vasomotor episodes. In agreement with this are the observations that serotonin rapidly disappears from the circulation following intravenous infusion[34] and that amounts in the order of 15 to 20 mg. per kg. must be administered to experimental animals to cause a detectable rise in circulating serotonin.[35] It may be necessary to devise a method for the measurement of unbound circulating serotonin to prove a relationship to the vasomotor disturbances.

OBSERVATIONS

It is apparent that carcinoid tumors are of the secreting or "endocrine" type, the production of serotonin being analogous to the production of epinephrine and nor-epinephrine by pheochromocytomas. These studies not only afford a basis for diagnosing and understanding this unusual condition but also focus attention on the possible importance of 5-hydroxyindole compounds in physiologic mechanisms and pathologic states.

Diagnosis of Malignant Carcinoid by Chemical Means. The greatly elevated urinary excretion of the serotonin metabolite 5-HIAA appears to be an invariable feature of malignant carcinoid and is diagnostic of this disorder. A preliminary survey of a variety of clinical conditions has failed to reveal other disorders with elevated 5-HIAA excretion.[36] Measurement of this substance in urine may be a more reliable test than needle biopsy of the liver, since in the patients in Cases III and IV the initial impression of liver biopsy material was "hepatoma." A simple qualitative test for 5-HIAA has been devised which can be carried out in a few minutes on a small amount of urine.[37] This test should prove useful as a screening procedure for malignant carcinoid.

It is not known whether all carcinoid tumors secrete serotonin or how small a mass of functioning tumor can be detected by the measurement of urinary 5-HIAA. The patient in Case VI did not have liver metastases and showed the smallest elevation of 5-HIAA excretion, which nevertheless was at least eight times that of normal. It is likely that carcinoid tumors can be diagnosed chemically at a time when the lesion is still resectable, but in such cases urinary excretion of 5-HIAA may be so low as to require use of the more sensitive quantitative assay.

Correlation of Clinical Manifestations with Abnormality of Tryptophan Metabolism. On the basis of clinical and pathologic data alone, it is reasonable to suspect a causal relationship between carcinoid tumors and the associated syndrome. The demonstration of a profound alteration in the metabolism of an essential amino acid in patients with this condition may provide a basis for understanding the complex pathophysiologic findings. A simplified diagrammatic comparison of tryptophan metabolism in normal subjects and carcinoid patients is shown in Figure 5. In normal subjects only about 1 per cent of dietary tryptophan is utilized in the 5-hydroxyindole pathway to serotonin-type compounds, and ade-

quate tryptophan is available for the formation of niacin and protein. In the "carcinoid syndrome" as much as 60 per cent of the daily intake of tryptophan may be diverted by the tumor into the serotonin pathway, leaving less of the amino acid available for the formation of niacin and protein. Hence one might expect symptoms in carcinoid patients attributable not only to excess serotonin production but also to niacin and protein deficiency, particularly during periods of prolonged anorexia and diarrhea. The two unidentified 5-hydroxyindoles found in carcinoid tumor and in the urine of carcinoid patients may also produce physiologic effects. These various factors will now be considered in respect to pathogenesis of the syndrome.

Serotonin excess: Diarrhea, bronchoconstriction and vasomotor disturbances are explicable as direct pharmacologic effects of serotonin. Its stimulant action on intestinal smooth muscle in various animal species is well known,[16] and increased motility of the intestine following serotonin administration has been observed in the anesthetized dog and rabbit.[38] Bronchoconstriction has been produced by administration of this agent to animals[39,40] and to patients with asthma.[41] Local erythema and obliteration of subcutaneous veins follow intradermal injection of serotonin in man.[42] Obliteration of subcutaneous veins was also observed in this laboratory during intravenous infusions of serotonin. Cutaneous flushing has been reported as a side effect in hypertenstive patients who were given serotonin infusions.[43] Although serotonin undoubtedly has a potent arteriolar vasoconstrictor effect in such preparations as the rabbit ear,[44] its action on systemic blood pressure is more complex.[16,43] In this laboratory depressor responses have been observed consistently in normotensive subjects infused with serotonin at a rate of 0.5 mg. per minute. Hence bronchoconstriction, intestinal hypermotility, flushing, superficial venoconstriction and hypotension which are found in the "carcinoid syndrome" have been produced in various experimental situations by injection of serotonin. It is of interest that reserpine,which has been shown to be a serotonin-releasing agent,[45] can produce most of these same effects.

Niacin deficiency: Although pellagra has been observed in patients with malignant carcinoid,[2,46] our patients exhibited none of the signs of niacin deficiency except diarrhea. Loss of nutrients from the intestinal tract in combination with a diminution in niacin production resulting from the abnormality of tryptophan metabolism should make these patients particularly prone to the development of pellagra. For this reason each of our patients was given vitamin supplements including niacin.

Protein deficiency: Weight loss and tissue wasting

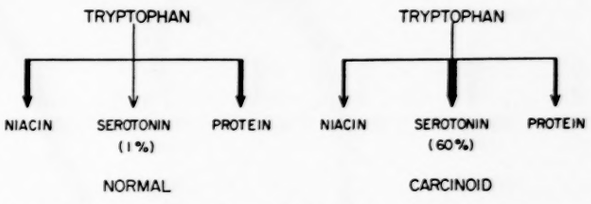

FIG. 5. Comparison of tryptophan metabolism in normal subjects and in patients with malignant carcinoid.

are prominent features of the "carcinoid syndrome." In addition, electrophoretic analyses indicated that three of our patients had slight hypoalbuminemia. Because of the abnormality in tryptophan metabolism, the minimal daily requirement of tryptophan for patients with the "carcinoid syndrome" may approach an amount which is several times the normal requirement. Hence decreased food intake or loss of nutrients by diarrhea which would not significantly affect protein synthesis in a normal subject can be expected to lead to negative nitrogen balance in a patient with malignant carcinoid.

Pathogenesis of Cardiac Lesions. Although no experimental evidence has appeared relating cardiac lesions to serotonin excess, this factor must be considered. As previously stated, a more generalized connective tissue disorder than that affecting the heart valves and endocardium is suggested by the presence of arthritic symptoms in some patients. The predisposition to involvement of the right portion of the heart may result from the following circumstances. Liver metastases have been found in all patients with cardiac lesions. This means that the liver, an organ normally capable of destroying serotonin, is now in effect secreting serotonin. The blood reaching the right portion of the heart may therefore be relatively rich in serotonin. However, blood entering the left portion of the heart may be somewhat depleted of free serotonin during passage through the lungs, a tissue also capable of destroying this amine. Supporting this hypothesis is the observation of extensive involvement of both the left and right-sided heart valves in a carcinoid patient in whom blood by-passed the lungs through a patent foramen ovale.[47] Failure

to demonstrate a difference in serotonin content of blood entering and leaving the lungs may again be ascribed to the inability of current methods to measure "free" serotonin.

The relative deficiency of tryptophan in these patients may also be a factor contributing to the development of cardiac lesions. Both factors, serotonin excess and tryptophan deficiency, should be investigated in further studies on this aspect of the syndrome.

Other Serotonin Relationships of Clinical Interest. It has been postulated that release of serotonin from the platelets is important in vasoconstriction during hemostasis.[48] The occurrence of thrombocytosis following administration of large amounts of serotonin to rats has been reported,[49] and serotonin has been invoked as a factor in clot retraction.[50] The carcinoid patients having a platelet serotonin excess exhibit no discernible hematologic abnormalities.

It has been suggested that serotonin may be important in brain function,[51,52] and evidence for such a relationship has been obtained recently.[53] With the possible exception of the patient in Case 1, the carcinoid patients showed no obvious mental abnormalities. If serotonin influences functions of the central nervous system, the absence of significant mental effects in these patients with marked hyperserotoninemia may be due to difficulty in the passage of this substance through the blood-brain barrier. Lysergic acid diethylamide (L.S.D.), a drug which antagonizes the action of serotonin on smooth muscle[54,55] and which produces artificial psychoses in man,[56] was administered to two of the carcinoid patients. In each case responsiveness to a normal dose was observed in terms of psychologic effects.[57] However, there were striking peripheral effects consisting of aggravation of flushing reactions and bronchospasm. Such effects have not been observed following administration of L.S.D. to normal subjects and were the reverse of what would be expected to follow administration of a serotonin antagonist to a patient with excess serotonin.

SUMMARY

1. Clinical and laboratory findings in six patients with the "malignant carcinoid syndrome" are presented.

2. The clinical findings in these patients confirmed previous descriptions of a syndrome manifested by vasomotor disturbances, chronic diarrhea, respiratory distress and valvular disease of the right portion of the heart. Two other consistent findings were arthritic symptoms and hypotension during flushes.

3. Using chemical methods, blood serotonin (5-hydroxytryptamine) levels of carcinoid patients were shown to range from 0.5 to 2.7 μg. per ml., compared to normal values of 0.1 to 0.3 μg. per ml. Analysis of a carcinoid tumor showed a serotonin content of 0.8 mg. per gm. and the presence of enzymes involved in the formation and destruction of serotonin.

4. Urinary excretion of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), ranged from 76 to 580 mg. per day in carcinoid patients as compared to 2 to 9 mg. per day in normal human subjects. Because of the extreme elevation of urinary 5-HIAA, its measurement provides a simple and rapid diagnosis of malignant carcinoid.

5. The precursor relationship of dietary tryptophan to 5-hydroxyindoles in man was demonstrated by a metabolic balance study and tracer studies on these patients. As much as 60 per cent of dietary tryptophan is converted to urinary 5-hydroxyindoles in this disorder. Normally only about 1 per cent of ingested tryptophan is metabolized in this manner.

6. Many of the manifestations of the "carcinoid syndrome" are apparently the result of serotonin excess, as has been suggested previously. However, the marked alteration of trytophan metabolism, resulting in a concomitant disturbance in niacin and protein production, should also be considered a contributing factor in the pathogenesis of this condition.

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REFERENCES

1. Biörck, G., Axen, O. and Thorson, A. Unusual cyanosis in a boy with congenital pulmonary stenosis and tricuspid insufficiency. Fatal outcome after angiocardiography. *Am. Heart J.*, 44: 143, 1952.
2. Thorson, A., Biörck, G., Björkman, G. and Waldenström, J. Malignant carcinoid of the small intestine with metastases to the liver, valvular disease of the right side of the heart (pulmonary stenosis and tricuspid regurgitation without septal defects), peripheral vasomotor symptoms, bronchoconstriction and an unusual type of cyanosis. A clinical and pathologic syndrome. *Am. Heart J.*, 47: 795, 1954.
3. Isler, P. and Hedinger, V. C. Metastasierendes Dünndarmcarcinoid mit schweren, vorwiegend das rechte Herz betreffenden Klappenfehlern und Pulmonalstenose—ein eigenartiger Symptomenkomplex? *Schweiz. med. Wchnschr.*, 83: 4, 1953.
4. Rosenbaum, F. F., Santer, D. C. and Claudon, D. B. Essential telangiectasia, pulmonic and tricuspid stenosis, and neoplastic liver disease. A possible new clinical syndrome. (*Abst.*) *J. Lab. & Clin. Med.*, 42: 941, 1953.
5. Branwood, A. W. and Bain, A. D. Carcinoid tumor of small intestine with hepatic metastases, pulmonary stenosis, and atypical cyanosis. *Lancet*, 2: 1259, 1954.
6. Jenkins, J. S. and Butcher, P. J. A. Malignant argentaffinoma with cyanosis and pulmonary stenosis. *Lancet*, 1: 331, 1955.
7. Bean, W. B., Olch, D. and Weinberg, H. B. The syndrome of carcinoid and acquired valve lesions of the right side of the heart. *Circulation*, 12: 1, 1955.
8. Rapport, M. M., Green, A. A. and Page, I. H. Partial purification of the vasoconstrictor in beef serum. *J. Biol. Chem.*, 174: 735, 1948.
9. Rapport, M. M. Serum vasoconstrictor (serotonin). v. The presence of creatinine in the complex. A proposed structure of the vasoconstrictor principle. *J. Biol. Chem.*, 180: 961, 1949.
10. Rand, M. and Reid, G. Source of serotonin in serum. *Nature, London*, 168: 385, 1951.
11. Vialli, M. and Erspamer, V. Cellule enterocromaffini e cellule basigranulose acidofile nei vertebrati. *Zschr. Zellforsch*, 19: 743, 1933.
12. Vialli, M. and Erspamer, V. Sulle reazoni chimiche colorate dell'enteramine. i. Ricerche su estretti acetonici di mucosa gastro-intestinale. *Arch. di sc. biol.*, 28: 101, 1942.
13. Erspamer, V. and Asero, B. Identification of enteramine, the specific hormone of the enterochrommaffin cell system, as 5-hydroxytryptamine. *Nature, London*, 169: 800, 1952.
14. Lembeck, F. 5-Hydroxytryptamine in a carcinoid tumor. *Nature, London*, 172: 910, 1953.
15. Ratzenhofer, M. and Lembeck, F. Über den Gehalt an 5-Oxytryptamin in Carcinoiden des Darmtraktes. *Ztschr. f. Krebsforsch.*, 60: 169, 1954.
16. Erspamer, V. Pharmacology of indolealkylamines. *Pharmacol. Rev.*, 6: 425, 1954.
17. Page, I. H. Serotonin (5-hydroxytryptamine). *Physiol. Rev.*, 34: 563, 1954.
18. Udenfriend, S. and Titus, E. The 5-Hydroxyindole Pathway of Tryptophan Metabolism. Amino Acid Metabolism, p. 945. The Johns Hopkins Press. Baltimore, 1955.
19. Sjoerdsma, A., Smith, T. E., Stevenson, T. D. and Udenfriend, S. Metabolism of 5-hydroxytryptamine (serotonin) by monamine oxidase. *Proc. Soc. Exper. Biol. & Med.*, 89: 36, 1955.
20. Sjoerdsma, A. and Udenfriend, S. Studies on indole metabolism in patients with malignant carcinoid (argentaffinoma). *J. Clin. Investigation*, 34: 914, 1955.
21. Udenfriend, S., Weissbach, H. and Clark, C. T. The estimation of 5-hydroxytryptamine (serotonin) in biological tissues. *J. Biol. Chem.*, 215: 337, 1955.
22. Udenfriend, S., Titus, E. and Weissbach, H. The identification of 5-hydroxy-3-indoleacetic acid in normal urine and a method for its assay. *J. Biol. Chem.*, 216: 499, 1955.
23. Udenfriend, S., Titus, E., Weissbach, H. and Peterson, R. E. Biogenesis and metabolism of 5-hydroxyindole compounds. *J. Biol. Chem.* (In press).
24. Reifenstein, E. C., Albright, F. and Wells, S. L. The accumulation, interpretation, and presentation of data pertaining to metabolic balances, notably those of calcium, phosphorus, and nitrogen. *J. Clin. Endocrinol*, 5: 367, 1945.
25. Ahrens, E. H., Dole, V. P. and Blankenhorn, D. H. The use of orally-fed liquid formulas in metabolic studies. *Am. J. Clin. Nutrition*, 2: 336, 1954.
26. Bumpus, F. M. and Page, I. H. Serotonin and its methylated derivatives in human urine. *J. Biol. Chem.*, 212: 111, 1955.
27. Clark, C. T., Weissbach, H. and Udenfriend, S. 5-hydroxytryptophan decarboxylase: preparation and properties. *J. Biol. Chem.*, 210: 139, 1954.
28. Mitoma, C., Weissbach, H. and Udenfriend, S. Formation of 5-hydroxytryptophan from tryptophan by chromobacterium violaceum. *Nature, London*, 175: 994, 1955.
29. Rose, W. C. Amino acid requirements of man. *Federation Proc.*, 8: 546, 1949.
30. Rose, W. C., Lambert, G. F. and Coon, M. J. The amino acid requirements of man. vii. General procedures; the tryptophan requirement. *J. Biol. Chem.* 211: 815, 1954.
31. Pernow, B. and Waldenström, J. Paroxysmal flushing and other symptoms caused by 5-hydroxytryptamine and histamine in patients with malignant tumors. *Lancet*, 2: 951, 1954.
32. Langemann, H. Personal communication.
33. Udenfriend, S. and Weissbach, H. Studies on serotonin (5-hydroxytryptamine) in platelets. *Federation Proc.*, 13: 412, 1954.
34. Twarog, B. M. and Page, I. H. Serotonin content of some mammalian tissues and urine and a method for its determination. *Am. J. Physiol.*, 175: 157, 1953.
35. Bogdanski, D. F. and Udenfriend, S. Unpublished observations.
36. Haverback, B. J. and Sjoerdsma, A. Unpublished observations.
37. Sjoerdsma, A., Weissbach, H. and Udenfriend, S. Simple test for diagnosis of metastatic carcinoid (argentaffinoma). *J. A. M. A.*, 159: 397, 1955.
38. Freyburger, W. A., Graham, B. E., Rapport, M. M., Seay, P. H., Govier, W. M., Swoap, O. F. and Van Der Brook, M. J. The pharmacology of 5-hydroxytryptamine (serotonin). *J. Pharmacol. & Exper. Therap.*, 105: 80, 1952.

39. Reid, G. and Rand, M. Pharmacological actions of 5-hydroxytryptamine (serotonin, thrombocytin). *Nature, London*, 169: 801, 1952.
40. Comroe, J. H., Van Lingen, B., Stroud, R. C. and Roncoroni, A. Reflex and direct cardiopulmonary effects of 5-OH-tryptamine (serotonin). *Am. J. Physiol.*, 173: 379, 1953.
41. Herxheimer, H. Influence of 5-hydroxytryptamine on bronchial function. *J. Physiol.*, 122: 490, 1953.
42. Reid, G. Circulatory effects of 5-hydroxytryptamine. *J. Physiol.*, 118: 435, 1952.
43. Page, I. and McCubbin, J. W. The variable arterial pressure response to serotonin in laboratory animals and man. *Circulation Res.*, 1: 354, 1953.
44. Ginzel, K. H. and Kottegoda, A. R. A study on the vascular actions of 5-hydroxytryptamine, tryptamine, adrenaline and noradrenaline. *Quart. J. Exper. Physiol.*, 38: 225, 1953.
45. Pletscher, A., Shore, P. A. and Brodie, B. B. Serotonin release as a possible mechanism of reserpine action. *Science*, 122: 374, 1955.
46. McNeely, R. G. D. and Jones, N. W. Secondary pellagra caused by multiple argentaffin carcinoma of the ileum and jejunum. *Gastroenterology*, 6: 443, 1946.
47. Rich, A. R. Personal communication.
48. Zucker, M. B. Platelet agglutination and vasoconstriction as factors in spontaneous hemostasis in normal, thrombocytopenic, heparinized and hypoprombinemic rats. *Am. J. Physiol.*, 148: 275, 1947.
49. Hedinger, V. C. and Langemann, H. Ausgesprochene Thrombocytose bei Ratten unter Behandlung mit 5-Oxytryptamin. *Schweiz. med. Wchnschr.*, 85: 368, 1955.
50. Fenichel, R. L. and Seegers, W. H. Bovine platelets, serotonin and retraction of bovine plasma clots. *Am. J. Physiol.*, 181: 19, 1955.
51. Woolley, D. W. and Shaw, E. A biochemical and pharmacological suggestion about certain mental disorders. *Proc. Nat. Acad. Sc.*, 40: 228, 1954.
52. Gaddum, J. H. Drugs Antagonistic to 5-Hydroxytryptamine. Ciba Foundation Symposium on Hypertension, p. 75. Boston, 1954. Little, Brown & Co.
53. Shore, P. A., Silver, S. A. and Brodie, B. B. Interaction of reserpine, 5-hydroxytryptamine (serotonin) and lysergic acid diethylamide in the central nervous system. *Science*, 122: 284, 1955.
54. Gaddum, J. H. Antagonism between lysergic acid diethylamide and 5-hydroxytryptamine. *J. Physiol.* 121: 15, 1953.
55. Gaddum, J. H., Hebb, C. O., Silver, A. and Swan, A. A. A. 5-hydroxytryptamine: pharmacological action and destruction in perfused lungs. *Quart. J. Exper. Physiol.*, 38: 255, 1953.
56. Stoll, W. A. Lysergsäure-diäthylamid, ein Phantastikum aus der Mutterkorngruppe. *Schweiz. Arch. f. Neurol. u. Psychiat.*, 60: 279, 1947.
57. Sjoerdsma, A., Kornetsky, C. H. and Evarts, E. Action of lysergic acid diethylamide (LSD) in patients with hyperserotonemia. *Arch. Neurol. & Psychiat.* In press.

Essential Cryoglobulinaemia*

Review of the Literature and Report of a Case Treated with ACTH and Cortisone

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THE objects of this report are (1) to record certain observations on a patient with "essential cryoglobulinaemia" who was treated for prolonged periods with various hormones before finally succumbing to his illness, (2) to outline studies carried out on the abnormal protein to determine something of its nature and (3) to attempt a clarification of its role in the observed clinical phenomena.

In 1933 Wintrobe and Buell[1] first described a cold-precipitable globulin in the blood of a patient suffering from multiple myeloma. This observation was soon repeated by numerous investigators,[2–9] and in 1947 the term cryoglobulin was introduced by Lerner and Watson[10] to describe proteins with this abnormal physical characteristic.

The presence of small quantities of cryoglobulin may be demonstrated not infrequently in the sera of patients with numerous disease states. In a study of 121 patients with miscellaneous diseases Lerner, Barnum and Watson[11] found thirty-one with cryoglobulin in the serum. Barr et al.[12] made similar observations in 1950. This protein has been noted in multiple myeloma,[10–23,38,78,79] kala-azar,[6,24] disseminated lupus erythematosus,[12] rheumatoid arthritis,[5] periarteritis nodosa,[9,25,36] subacute bacterial endocarditis,[26] coronary artery disease,[27] lymphosarcoma,[28] lymphatic leukaemia,[29,80] polycythaemia rubra vera,[30] hepatic cirrhosis[31,81] and numerous other diseases.[6,11,12] Most cases showed but a small and therefore clinically unimportant quantity of cryoglobulin. Rarely was the cryoglobulin present in such large amounts as to be directly responsible for clinical signs and symptoms. In most of the instances when it was so responsible, the cryoglobulinaemia was a manifestation of multiple myeloma. Of the remainder, there have been four cases reported[12,32–34] in which no underlying disease entity could be found to explain the presence of the cryoglobulinaemia. The designation "essential cryoglobulinaemia" has been advanced to describe these cases in which the cause remains obscure.

The manifestations of cryoglobulinaemia (either "essential" or secondary) include cold sensitivity, atypical Raynaud's phenomenon, purpura, stomatitis, bleeding from nose and mouth, retinal haemorrhages, mottling of the lower extremities, dyspnoea, cyanosis, abdominal distress, diarrhoea, melaena and deafness. Gangrene in the extremities has been reported on four occasions.[16,34–36] Multiple arterial and venous occlusions involving most of the vessels in the body have been described recently by Cugudda.[16] Pulmonary vascular sclerosis due to the deposition of cryoglobulin in the pulmonary arterial tree was recorded recently by Muirhead et al.[37]

The age and sex incidence may be of some interest. A review of thirty-nine reported cases of patients with large concentrations of cryoglobulin (both "essential" and secondary) has revealed a range in ages between thirty-three and seventy-seven years, with the largest proportion (seventy-five per cent) falling in the sixth and seventh decades of life. There were twenty-eight males and eleven females in this group.

Unusual results with two therapeutic agents have come to our attention since the completion

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of the study herein outlined and may be briefly noted. In a case of polycythaemia rubra vera with cryoglobulinaemia Israels and Kilgore[30] found that radioactive phosphorus (P^{32}) therapy caused the cryoglobulin to disappear. Concomitantly, there was a reduction in the concentration of gamma globulin, as well as the expected haematologic response. Thereafter there was a gradual re-elevation of the gamma globulin but the presence of cryoglobulin could not again be demonstrated. In a case of multiple myeloma with cryoglobulinaemia Osserman et al.[78,79] found 6-mercaptopurine to cause temporary disappearance of this protein, with an associated reduction in the gamma globulin. Following withdrawal of therapy due to leukopenia the cryoglobulin again made its appearance.

Although reference to hormone therapy in cryoglobulinaemia has been made occasionally,[17,32,33,38,80] no long-term study of the effects of such therapy on clinical state and serum proteins in this condition has come to our notice. The ACTH and cortisone therapy in the patient herein reported was carried on for prolonged periods, thus providing opportunity for detailed observations.

CASE REPORT

W. K. S., a sixty-three year old man, was referred to Sunnybrook (D.V.A.) Hospital, Toronto, on November 11, 1953, and died on July 30, 1954. His chief complaints on admission were a blue and painful left great toe, chest pain and a haemorrhagic rash. His past illnesses included left-sided pleurisy in 1919, followed by chronic cough with some sputum and dyspnoea on effort; in 1938 he had had pneumonia.

Symptoms directly referable to the condition from which he finally died had their onset about twenty-five years before death. At that time the patient began to note that, on exposure to cold, blue tender spots would appear occasionally on his ears. These would take about two weeks to disappear. Over the years he continued to show these lesions without much change. Then, about ten years before death, the patient began to feel chilled on exposures to cold and experienced a true rigor, followed by a period of feverishness. Within twenty-four hours red and purple spots varying from 1/16 to 1/2 inch in diameter would appear on his legs. These lesions were frequently confluent in some areas. The large purple spots were tender, and usually ulcerated and bled. Healing required several weeks and permanent areas of mottled brown pigmentation, or circular depressed scars, remained.

The periodic character of these lesions was remarkable in that only occasional exposures to cold resulted in their appearance. After each subjection to low temperature resulting in manifestations no further symptoms or signs would occur on further exposure to cold for a period of about two months. (This interval, however, gradually decreased thereafter.) They occurred mainly in the winter but would also appear after cool damp summer evenings on the bowling green. Over the years these manifestations gradually became more pronounced and more frequent, and there was an increasing permanent blotchy brown pigmentation of the lower extremities.

In 1951 the patient experienced intermittent bouts of crampy lower abdominal pain of moderate severity. These persisted for several weeks and were associated with diarrhoea and tarry stools. A thorough gastrointestinal investigation was negative. During one particularly severe attack appendectomy was performed. The appendix was normal and no cause for the pain, which recurred postoperatively, was discovered.

About this time the patient began to note pain, numbness and tingling, and cyanosis of his fingertips on exposure to cold.

Early in October, 1953, his right great toe became cold, painful and blue within a few hours. (The patient remained thereafter in a warm environment continuously; his subsequent manifestations thus had no apparent relationship to exposure to cold.) He was put to bed, given oral priscoline,® and local heat was applied to his right foot. Within five days his toe appeared normal. Late in October (two weeks prior to admission) his left great toe became similarly affected and similar management resulted in only slight improvement. One week prior to admission he had a true rigor; within twenty-four hours a purpuric rash appeared on the ears, upper extremities and lower abdomen. The same day he passed several tarry, loose stools. That evening he suddenly began to cough and a severe right axillary pleuritic pain developed; the next morning he coughed up several dark red blood clots.

Two days before admission he suffered a recurrence of his rigors, purpura, haemoptysis and right-sided pleuritic pain.

Another interesting point in the history was that the patient had noted increasing deafness during the past ten years, more pronounced in winter than in summer. This had become much more severe in the five-month period before admission.

There was no personal or family history of allergy, and no family history of bleeding tendencies.

Physical examination on admission revealed a plethoric, slightly dyspnoeic, slightly cyanotic, moderately obese man. He did not appear acutely ill. Ophthalmoscopic examination was not remarkable. Moderate bilateral mixed deafness was noted. Scattered rales were present in both lung fields, and a pleural friction rub was heard in the right axilla. The heart was normal. Blood pressure was 140/80. The abdomen was obese, and the liver, spleen and

FIG. 1. Patient W. K. S., showing dry gangrene of toes with mottled pigmentation of the skin of the lower extremities.

FIG. 2. Posteroanterior chest film taken December 4, 1953, demonstrating a linear plate of atelectasis in right middle lobe following an episode of pleuritic pain and haemoptysis. Similar radiologic lesions were observed elsewhere after other such episodes also clinically believed to be pulmonary infarction.

kidneys were not palpable. There was no ascites. Both lower extremities were covered by areas of mottled brown pigmentation, most prominent below the knees. On the medial aspect of the thighs were numerous circular depressed scars. There was a small shallow haemorrhagic ulcer about ⅜ inch in diameter on the left calf. The left great toe was blue, cold, painful and tender. The left dorsalis pedis pulse was strong and easily palpable, and the foot was warm. The left posterior tibial pulse could not be felt. On the right side the posterior tibial pulse was normal but the dorsalis pedis pulse was not palpable. This foot was also warm. There was no clinical evidence of thrombophlebitis in the lower extremities. There were many dilated venules on the nose and face but there were no arterial cutaneous "spiders." Many purpuric spots on the ears, face and upper extremities were noted. There was no superficial lymphadenopathy. Rectal and neurologic examinations were normal.

Although numerous possible diagnoses were suggested on admission it was not until almost one month later that the patient was discovered to have cryoglobulinaemia. An extensive investigation (*v. seq.*) failed to reveal an underlying cause to explain the presence of this abnormal protein.

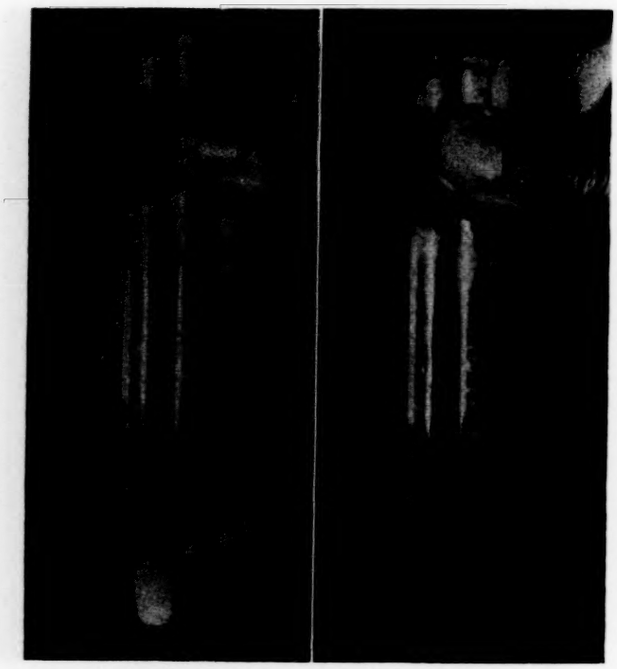
Following admission the patient's course was poor. Despite intra-arterial administration of priscoline and heat to the body, dry gangrene and mummification occurred in the left great toe and then the second toe. (Fig. 1.) On November 25, 1953, he experienced a true rigor, and within twenty-four hours a petechial rash appeared on the hips and back. He continued to have repeated rigors, fever and crops of purpuric spots on the face, trunk and extremities. Severe haemorrhagic stomatitis and pharyngitis developed. On December 4th he experienced severe left-sided pleuritic pain associated with cough and haemoptysis; and on December 10th right axillary pleuritic pain developed, also with cough and haemoptysis. (Fig. 2.) During this period there was tenderness behind the left knee and the left calf was warmer than the right. Although there was no calf tenderness, no measurable swelling and Homans' sign was negative, it was considered that the patient had thrombophlebitis with multiple pulmonary emboli. At the same time the patient's deafness became much more marked, and epistaxis, extreme dyspnoea and cyanosis developed. By the morning of December 11th the patient appeared dangerously ill.

The haemoglobin, erythrocyte count, haematocrit, leukocyte count, differential white cell count, eosinophil count, platelet count and smear were all normal on repeated examination. The bleeding time, clotting time, clot retraction time, prothrombin time and capillary fragility test were also repeatedly normal. Bone marrow was aspirated on two occasions; the marrow elements were unremarkable, in particular there was no increase in plasma cells or megakaryocytes. Urinalyses were negative to routinely tested abnormal constituents. There was no proteinuria and, prior to the hormone therapy, no glycosuria.

Fasting blood sugars varied between 80 and 94 mg. per cent. The serum non-protein nitrogen, cholesterol, uric acid, calcium and the Congo red test were all within normal ranges.

The presumptive Kahn test was negative, as was the direct Coombs test. Cold agglutinins were 1:8 (normal). The anticomplementary titre was 1:512. This remarkably high titre was shown eventually to reside entirely in the cryoglobulin fraction.

When the serum was placed in the refrigerator a



3A 3B

FIG. 3. A, serum when cooled to 5°C. A gelatinous precipitate is present which appeared within two minutes' cooling. B, sample of serum rewarmed to 37°C. The cryoglobulin has redissolved.

considerable precipitation occurred within 120 seconds, and this precipitate promptly dissolved on rewarming the specimens to 37°C. (Fig. 3.) This precipitate soon formed an elastic translucent gel. It resided in the euglobulin fraction by the sodium sulphite precipitation technic.[39] By the sodium sulphate precipitation technic[42,43] the cryoglobulin precipitated in the 8 to 17 per cent range. By filter paper electrophoresis (Fig. 4) and free electrophoresis (micro-Antweiler) (Fig. 5) the cryoglobulin was found to migrate in the gamma globulin fraction. The concentration of cryoglobulin was determined in the following manner: Venous blood was aspirated and maintained at body temperature until the clot retracted; the serum was then removed, divided into two samples and maintained at 3°C. for twenty-four hours. One of these specimens was then warmed to 37°C. (at which point the cryoglobulin completely redissolved) and its serum proteins were then determined. From the second (cold) sample, the supernatant fluid was removed (from above the precipitated cryoglobulin) and serum proteins were determined on this supernatant fluid. The differences between the globulin determinations of the two fluids represented the amount of cryoglobulin; this varied between 0.6 to 1.6 gm./100 cc. throughout the patient's hospitalization. (Fig. 6.)

Sedimentation rates, serum mucoprotein (as tyrosine mg./100 cc., normals 2.6 to 3.4 mg./100 cc.)[40] and fibrinogen estimations[44] were carried out regularly and are recorded in Figure 6. While the patient was receiving duracton,* fasting blood sugars,[45] quantitative and qualitative urine sugars, qualitative urine acetone, carbon dioxide combining power, 17-ketosteroid excretion[46] and total eosinophil counts were determined as recorded in Figure 7.

No characteristic L.E. cells were found. However, when the test was performed at room temperature (21°C.) or chilled at 5°C., an unusual picture was produced. (Fig. 8.) Masses of homogeneous light blue staining material were seen lying free among the cells. This material was actually the precipitated cryoglobulin.[41] In addition, many polymorphonuclear leukocytes were seen which contained large inclusions in their cytoplasm. In some the inclusion was a blue homogeneous material which was also cryoglobulin. The process is one of phagocytosis of the precipitated cryoglobulin, and the term cryoglobulin inclusion cell has been advanced to describe these leukocytes.[41] Some of the inclusions appear colourless, perhaps due to redissolving of the cryoglobulin at room temperature.

Electrocardiograms were repeatedly normal. Chest x-rays revealed pulmonary lesions compatible with recurrent infarction. (Fig. 2.) A roentgenographic bone survey revealed normal skull, ribs, vertebrae and long bones. No osteoporosis was noted.

Response of Hands to Cold Water. When one of the patient's hands was placed in water at 10°C., the entire hand became very pale and cyanotic. There was an erythematous border at the proximal edge of the cyanotic skin. The hand was removed from the water after sixty seconds. The erythema then irregularly invaded the cyanotic area until the entire hand was intensely erythematous. This erythema subsided after fifteen minutes. The changes were localized to the exposed areas only. When two fingers alone were placed in the cold water there were no observed changes in any of the other digits. During these experiments the patient complained of some pain and tingling in the exposed fingers.

Response of Skin to Exposure to Ice. A cube of ice was placed on the skin of the abdomen for sixty seconds. An erythematous, raised wheal 2 inches in diameter appeared in two minutes, and faded after fifty minutes.

Effect of Hormone Therapy on Clinical State and Serum Proteins (Figs. 6 and 7). *First period* (*December 11 to 30, 1953*): ACTH therapy was first initiated (for reasons discussed later) on December 11, 1953, (15 units of Armour ACTH administered intravenously in 500 cc. of 5 per cent glucose in water over twelve-hour periods daily). The effect on the clinical condition was striking. Just prior to the institution of this therapy the patient appeared to be in a terminal state. However, within twenty-four hours his chills and fever subsided,

* Nordic Biochemicals Ltd., Montreal, Canada, brand of corticotrophin (ACTH) with carboxymethylcellulose (to prolong its action).

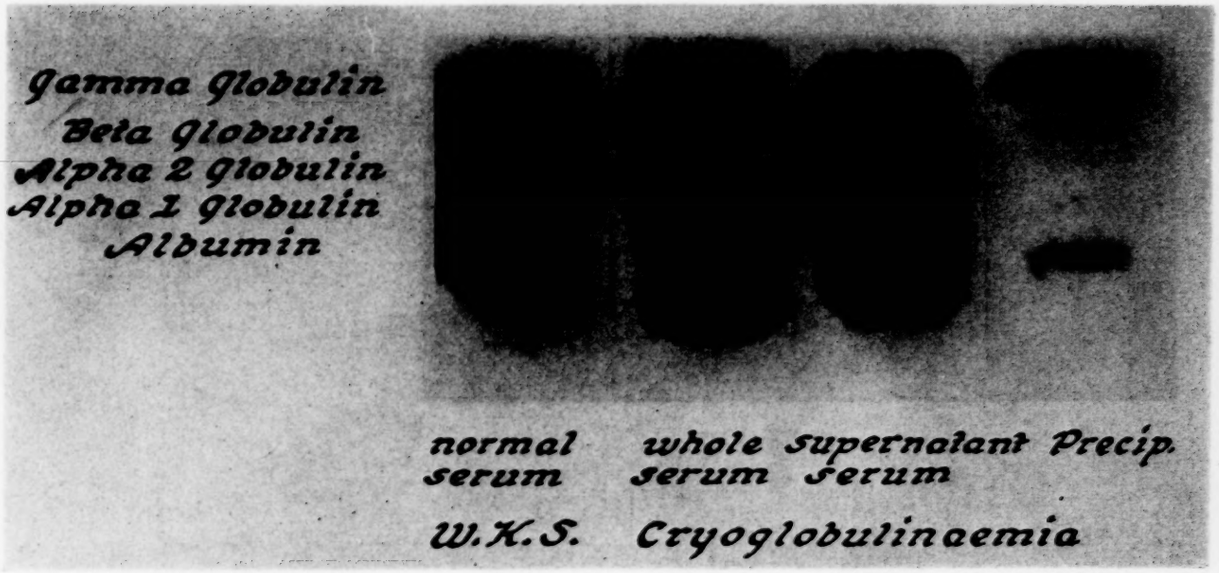


FIG. 4. Filter paper electrophoresis determinations showing the cryoglobulin to migrate in the gamma globulin fraction. Increases in alpha-1, alpha-2 and beta globulin are incidentally noted.

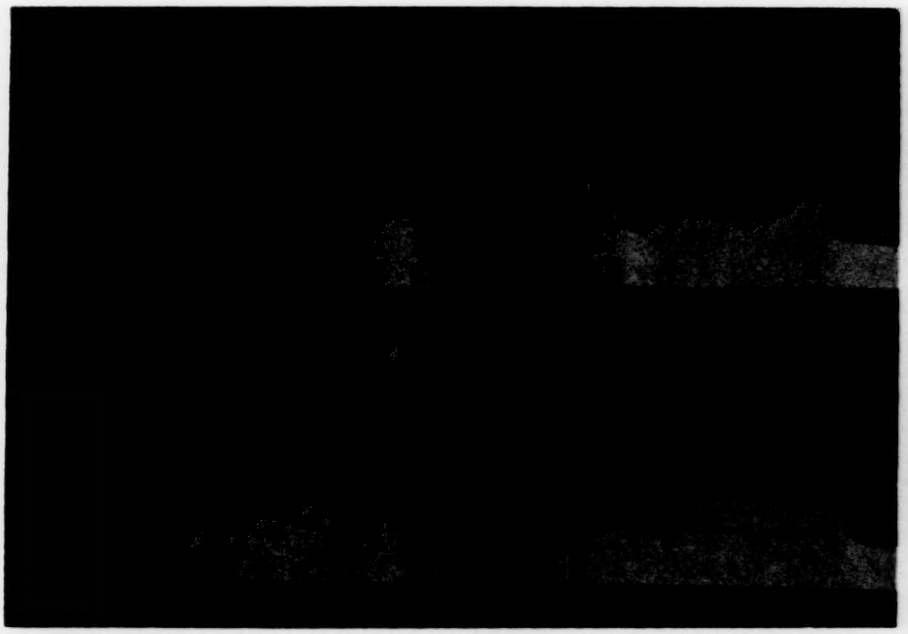


FIG. 5. Free electrophoresis (micro-Antweiler) determinations confirm the gamma globulin migration of the cryoglobulin. In addition, there appears to be an unstable beta lipoprotein in association with the cryoglobulin (see text). Whole serum: albumin 23.4 per cent, alpha-1 globulin 7.7 per cent, alpha-2 globulin 16.2 per cent, beta globulin 8.7 per cent, gamma globulin 36.9 per cent, others 6.9 per cent. Supernatant serum (removed at 10°c.): albumin 28.9 per cent, alpha-1 globulin 8.3 per cent, alpha-2 globulin 20.4 per cent, beta globulin 16.0 per cent, gamma globulin 20.8 per cent, others 5.6 per cent.

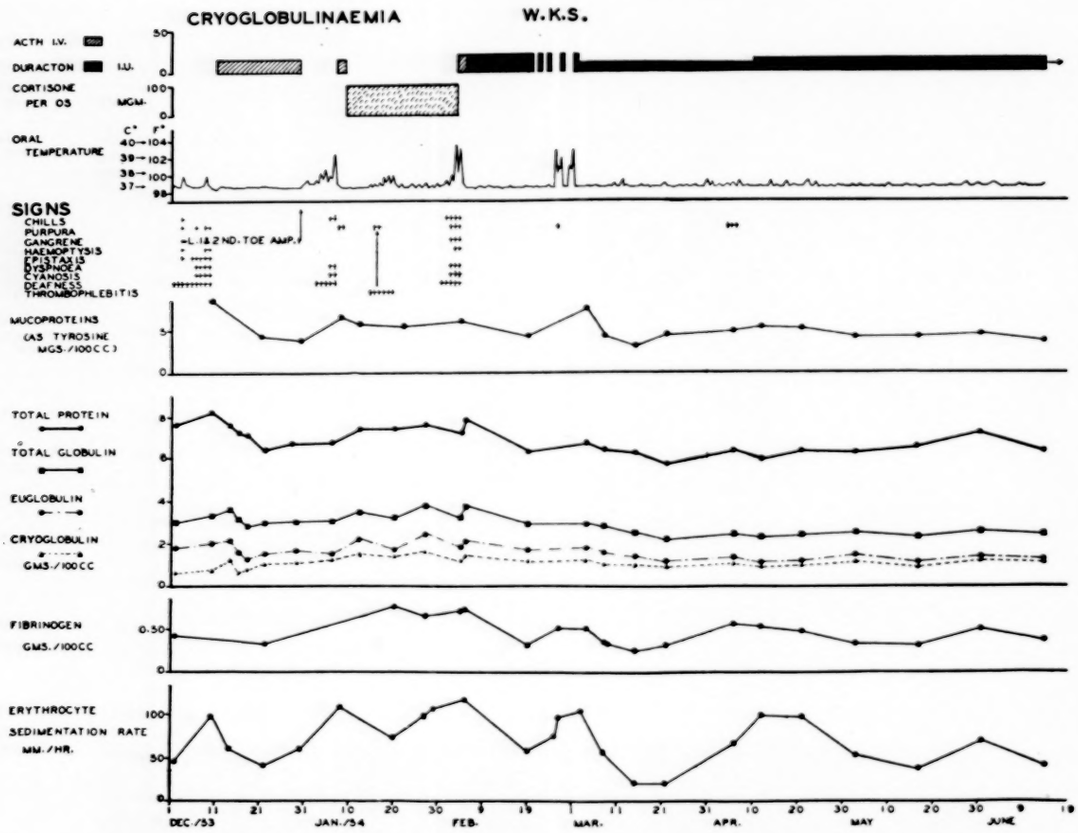


FIG. 6. Graph showing effects of ACTH and cortisone therapy on clinical state, serum proteins and erythrocyte sedimentation rate (during first hospitalization only). The serum proteins here recorded were determined by the sodium sulphite precipitation technic,[39] while the quantitative estimation of cryoglobulin was carried out as noted in the text. It should be noted that the temperature chosen at which the cryoglobulin was separated (i.e., 3°C.) was arbitrary, and thus quantitative results are scarcely comparable with those of other laboratories where the protein was separated at other temperatures. Mucoprotein estimations (as tyrosine mg. per cent) were performed by the method of Winzler et al.[40]

his deafness improved, and his dyspnoea, cyanosis, stomatitis and epistaxis all cleared and no further purpura appeared. The patient looked and felt well. He had no further manifestations of his disease while he was maintained on ACTH during this first period.

The concentration of the (electrophoretic) gamma globulin was decreased within one day of starting ACTH therapy and remained significantly diminished while this treatment was maintained. (Fig. 10.) However, there was no striking alteration in the cryoglobulin fraction of the gamma globulin. The same is naturally true for the cryoglobulin fraction of the euglobulin (sodium sulphite technic, Fig. 6).

The erythrocyte sedimentation rate dropped from 98 to 42 mm./hr. during therapy but increased again to 70 mm./hr. on December 30th, the day ACTH was discontinued. Fibrinogen dropped from 0.52 to 0.42 gm./100 cc., and mucoproteins from 8.6 mg./100 cc. to 3.7 mg./100 cc. while the patient was receiving ACTH therapy.

Second period (*December 30, 1953 to January 7, 1954*): On December 30th amputation of the left great and second toes was performed, and the ACTH was discontinued. For the pathologic findings, see Figure 9. However, on January 1st the patient began to run a swinging fever which increased in amplitude. He again became deaf, experienced malaise, rigors and purpura, and was again very dyspnoeic and cyanotic. His legs were oedematous but there was no redness or tenderness, and Homans' sign was negative. By January 7th the patient again looked very seriously ill.

During this period also there was little change in the quantity of cryoglobulin. Marked increases in the sedimentation rate and serum mucoproteins were noted.

Third period (*January 7 to February 4, 1954*): On January 7th, because the patient again appeared desperately ill, intravenous ACTH was reinstituted at the previous dosage. As before, within twenty-four hours he felt well, his fever and purpura subsided, his dyspnoea and cyanosis disappeared, he no longer coughed up sputum, and his deafness improved. On

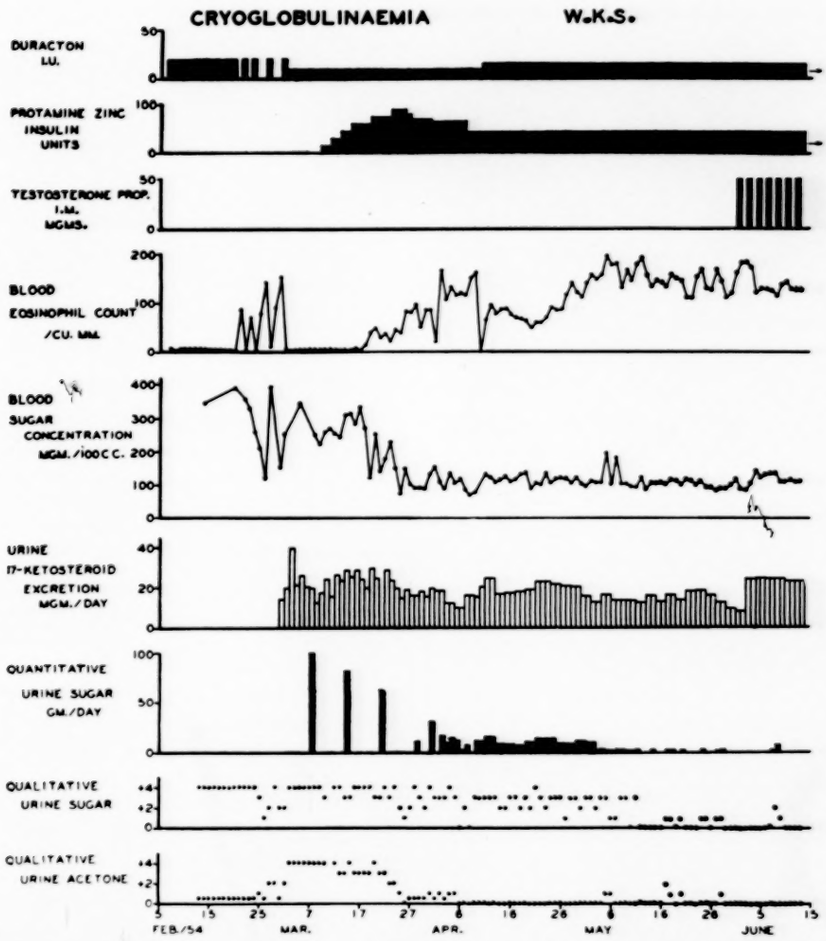


FIG. 7. Graph demonstrating effects of duracton, insulin, and later, testosterone on eosinophil counts, fasting blood sugar, urine sugar and acetone, and daily 17-ketosteroid excretion (during latter part of first hospitalization).

January 9th the ACTH was discontinued and was replaced by oral cortisone acetate, 100 mg. daily. While receiving cortisone the patient noted mild malaise and an increase in deafness. Then on January 14th a very severe deep and superficial thrombophlebitis of the right leg developed, with marked purpura mainly along the course of the long saphenous vein. He was treated with heparin and dicumarol; in ten days the thrombophlebitis subsided.

On January 30th his deafness became more marked. On February 1st, while still receiving daily cortisone, he had a true rigor followed by fever. Within the next three days he experienced many rigors and his temperature rose as high as 103.6°F. Numerous crops of purpuric spots appeared on the ears, trunk and extremities. The right great toe became blue, painful and tender overnight, and appeared gangrenous. Stomatitis with postnasal bleeding again developed. Dyspnoea and cyanosis returned in marked degree, and he coughed up large quantities of mucopurulent sputum. By February 4, 1954, the patient again appeared to be dangerously ill.

Although not markedly different from previous values, the cryoglobulin, euglobulin and total globulin figures were at their greatest height during this period. In addition, the sedimentation rate and fibrinogen and serum mucoprotein levels remained elevated.

Fourth period (February 4 to March 1, 1954): On February 4th the cortisone was discontinued and ACTH (Armour) was reinstituted (20 units daily intravenously over twelve-hour periods). Once more, within twenty-four hours he looked and felt well, and all of his manifestations subsided. The right great toe, which had appeared gangrenous, began to improve and finally only a small piece of skin was lost. After two days the intravenous ACTH therapy was changed to long-acting intramuscular ACTH (duracton), 20 units daily. He was well for two weeks but then noted polyuria, polydipsia and polyphagia.

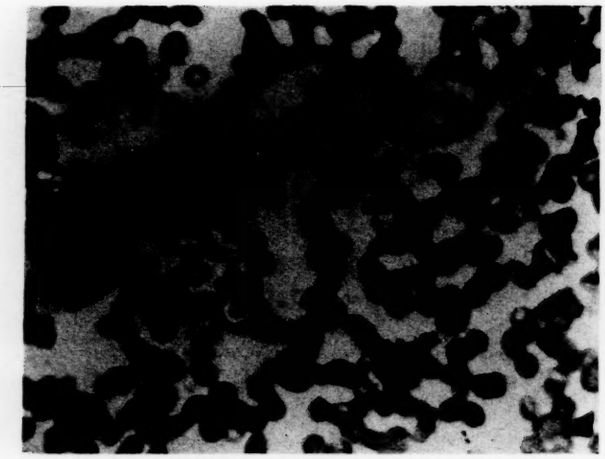


FIG. 8. Photomicrograph of venous blood preparation showing precipitated mass of cryoglobulin, and neutrophils containing cryoglobulin as phagocytized inclusions.

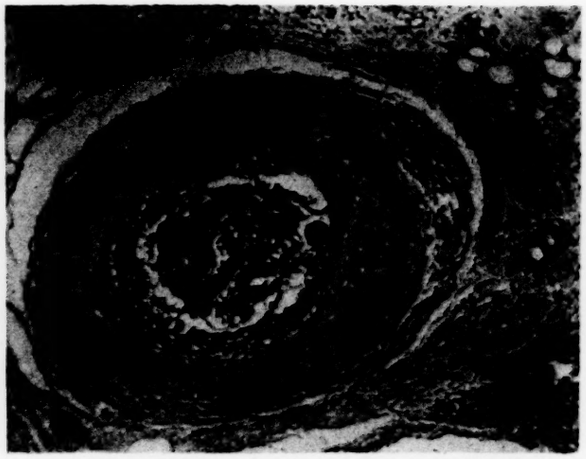


FIG. 9. Photomicrograph of digital artery (left great toe) amputated on December 20, 1953. Organized thrombus is noted. In other vessels (not shown) an eosinophilic coagulum was seen in the lumen. There was no histologic evidence of arteritis.

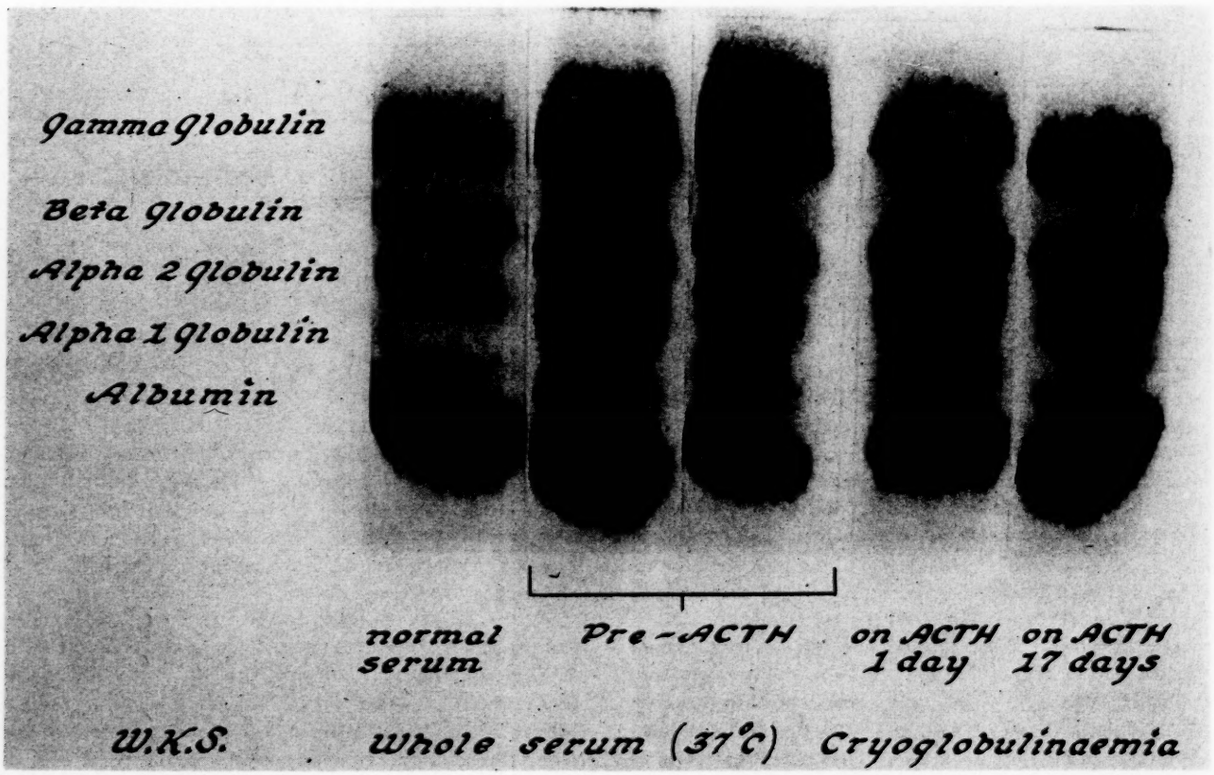


FIG. 10. Filter paper electrophoresis patterns during first period of ACTH therapy. There appeared to be an immediate fall in the gamma globulin, although this was not reflected in the cryoglobulin fraction (not shown) of the gamma globulin, which remained relatively constant. The gamma globulin remained decreased during this period.

It was soon determined that the patient had fasting hyperglycemia (360 mg./100 cc. on February 14th) and marked glycosuria. (Fig. 7.) Although a glucose tolerance test had never been performed, fasting blood sugars prior to hormone therapy had been normal, and there had been no glycosuria. Because of this evidence of steroid diabetes (Fig. 7) the dose of duracton was changed to 20 units every two days on February 20th, and then, after two such doses, to 20 units every three days. On the latter regimen, however, he experienced high fever on two occasions. Aside from malaise and the appearance of a few purpuric spots the patient remained free from major manifestations even while febrile.

During this fourth period there was again little change in the cryoglobulin, although the euglobulin did decrease slightly. The sedimentation rate and fibrinogen also dropped considerably until the period of intermittent duracton treatment, when both values increased. A similar trend was noted in the mucoprotein concentrations.

Fifth period (March 1 to April 10, 1954): Because of the recurrence of hyperpyrexia continuous duracton therapy was recommenced, 20 units being given the first day and 10 units daily thereafter. During the next five weeks the patient continued to feel well, although both legs became oedematous. On April 4th he noted new crops of purpura on the face and lower extremities, which persisted for the next week. Because of the reinstitution of daily duracton therapy the diabetes again became severe. It was decided, however, that it was absolutely essential to persist with the ACTH despite the diabetic state so produced. Therefore, the patient was started on a regimen of protamine zinc insulin, 15 units every morning, on March 9th and this was gradually increased until by March 23rd he was receiving 90 units of P.Z.I. every morning. However, after that date there were certain laboratory indications that duracton was becoming less effective. (Fig. 7.) The eosinophil counts were steadily becoming higher, and daily urine 17-ketosteroid excretion decreased gradually. His insulin requirement became less. This diminished effectiveness of the hormone was also demonstrated by a rising erythrocyte sedimentation rate with gradual elevation of fibrinogen and mucoprotein concentrations. (Fig. 6.) However, there was no evidence of clinical deterioration until April 4th, when the purpura recurred, as previously mentioned.

During this interval there were moderate reductions in the concentration of the total globulin and euglobulin; by contrast, there was only a slight decrease in the level of cryoglobulin so that it came to compose almost all of the euglobulin fraction. The (electrophoretic) gamma globulin also decreased, and the cryoglobulin came to constitute a greater percentage of it than previously.

Sixth period (April 10 to June 14, 1954): Because of the strong laboratory evidence of decreasing effectiveness of the then current dosage of duracton (10 units every morning) it was increased to 15 units every morning on April 10th. That same day amputation of the head of the left first metatarsal was performed in order that the previously open wound could be closed. His activity was thereafter very gradually increased until by May 10th he was able to spend much of his time walking around the ward. He continued to be well, with no further purpura. Some bilateral ankle oedema was still present.

Improvement continued, and consideration was given to discharging the patient home. However, on May 20th low back pain developed which increased in severity. Roentgenograms of the lumbar spine revealed osteroporosis and compression fractures of the superior surfaces of the first and second lumbar vertebrae. (Both the osteoporosis and fractures were recent, as they did not appear in the films of the lumbosacral spine taken on December 18, 1953, as part of a skeletal survey.) The patient was returned to bedrest with a fracture board. On May 31st testosterone propionate therapy was instituted, at a dosage schedule of 50 mg. intramuscularly every two days. Concomitant with these measures the back pain was greatly improved by June 2nd, and the patient gradually became ambulatory again. He was discharged feeling well on June 14, 1954. At that time his ankles were quite oedematous, particularly the right. He was maintained on duracton and protamine zinc insulin at the dosage schedule already described. The testosterone was discontinued on the day of discharge.

In this period there was no significant changes in the cryoglobulin concentration. The fibrinogen, sedimentation rate and mucoprotein levels decreased again. After initial responses to the increased dosage of ACTH, the eosinophil counts and urine 17-ketosteroid levels showed evidence of "escape" once more.

Seventh period (June 14 to July 30, 1954): The patient's feeling of well-being was maintained for three weeks following discharge. During this interval, however, the back pain recurred but was rapidly recontrolled by testosterone. The oedema of the lower extremities gradually ascended. On July 5th he was given a mercurial diuretic for the oedema, by then massive. A prompt diuresis occurred during which he felt weak and tremulous, and complained of blurring of vision, confusion and increased deafness. He was digitalized and returned to the hospital. Examination on this second admission revealed a drowsy, confused, apathetic and lethargic man. Examination of the chest, heart and abdomen yielded findings unchanged from previous occasions. Oedema of the back and lower extremities was massive. By contrast, the skin and subcutaneous tissue of the upper part of the body was markedly dehydrated. Signs of thrombophlebitis of the right leg were noted. There were purpuric lesions on the right pretibial region, thighs, buttocks and trunk.

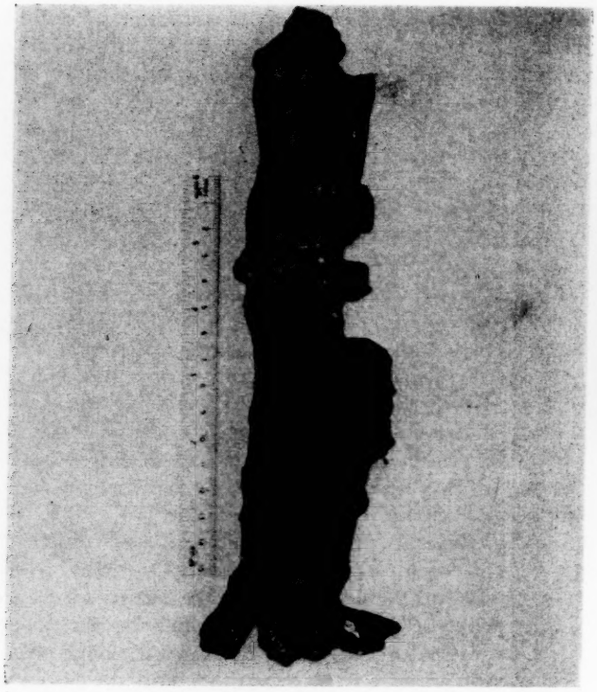


FIG. 11. Inferior vena cava removed at autopsy. There is an extensive adherent thrombosis extending from the veins of the right leg, almost as far proximal as the renal veins. Electrophoretic analysis of the thrombus revealed equal components migrating at the gamma globulin and fibrinogen positions, and the considerable remainder having the migration of haemoglobin.

It was thought that the patient had occlusion of the inferior vena cava, dating back several months, with subsequent oedema formation. It was not believed that a state of congestive heart failure was responsible. The cause of his mental state was not clear, although consideration was given to the possibility that the diuresis-induced dehydration in association with an already increased blood viscosity might lead to a greatly decreased cerebral blood flow. Investigation at this point revealed normal serum sodium, potassium, chloride and CO_2 combining power. The steroid diabetes was uncontrolled, however, with a fasting blood sugar level of 600 mg./100 cc. The serum non-protein nitrogen was 66 mg./100 cc. The haemoglobin was 112 per cent, the haematocrit was 50 per cent. A decholin circulation time showed an arm-to-tongue time of eighteen seconds, and a femoral vein-to-tongue time of thirty seconds. This was in favour of an inferior vena caval occlusion.

Duracton and insulin therapy was continued. The patient ran a peculiar course. On occasion, he was relatively bright and alert, and his hearing was good; at other times he was extremely lethargic, drowsy, confused, and his hearing poor. Terminally, bronchopneumonia and pulmonary oedema developed, and he died on July 30, 1954.

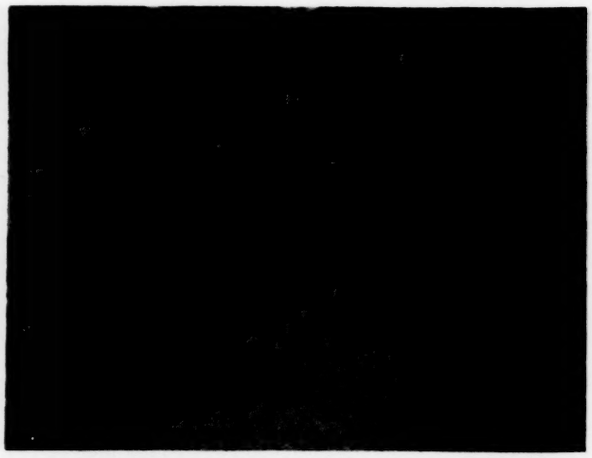


FIG. 12. Photomicrograph of pancreas showing an organized thrombus in a small artery. New and old thromboses were seen in similar-sized arteries in spleen and kidneys. No evidence of active or healed arteritis could be found, the vessel walls appearing intact.

During this period little change from previous determinations was observed in the various protein fractions.

Postmortem Findings (Figs. 11 and 12). Autopsy failed to disclose any underlying pathologic process of which the cryoglobulinaemia might have been symptomatic. There were two groups of lesions: one group secondary to the cryoglobulin, and a group secondary to prolonged ACTH therapy. The first group included multiple thromboses of small blood vessels in the skin and intestinal mucosa. Thrombosed and recanalized arteries were encountered in the spleen and pancreas. New and old infarcts were observed in the spleen and kidneys. Infarcts and resolving emboli were encountered in the lung. There was no suggestion of a healed or active arteritis in any of the arterial walls. Thrombosis of the superficial and deep veins of the right leg was demonstrated, with extension into the inferior vena cava proximally almost as far as the renal veins. The dorsalis pedis arteries were both patent but sclerotic. As a result of the ACTH therapy adrenocortical hyperplasia had been produced, with degranulation of the beta cells of the islets of Langerhans, severe generalized osteoporosis and atrophy of skin. Extensive Crooke's changes in the basophils of the pituitary were also noted. Other findings were chronic pulmonary emphysema, moderately advanced atherosclerosis of the aorta and moderate generalized atherosclerosis. Terminal events included pulmonary oedema and bronchopneumonia. There were no relevant gross or microscopic abnormalities in the brain.

SPECIAL STUDIES (IN VITRO)

Pertinent to Clinical Manifestations. Solubility of the protein (with reference to temperature and dilution):

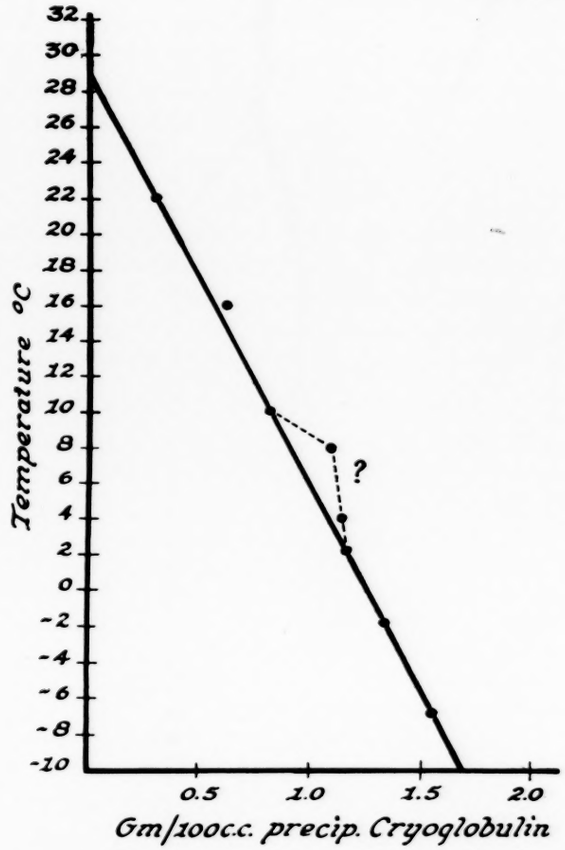


FIG. 13A. Patient W. K. S. Cryoglobulinaemia. Precipitation of cryoglobulin in a sample of patient's serum at various temperatures.

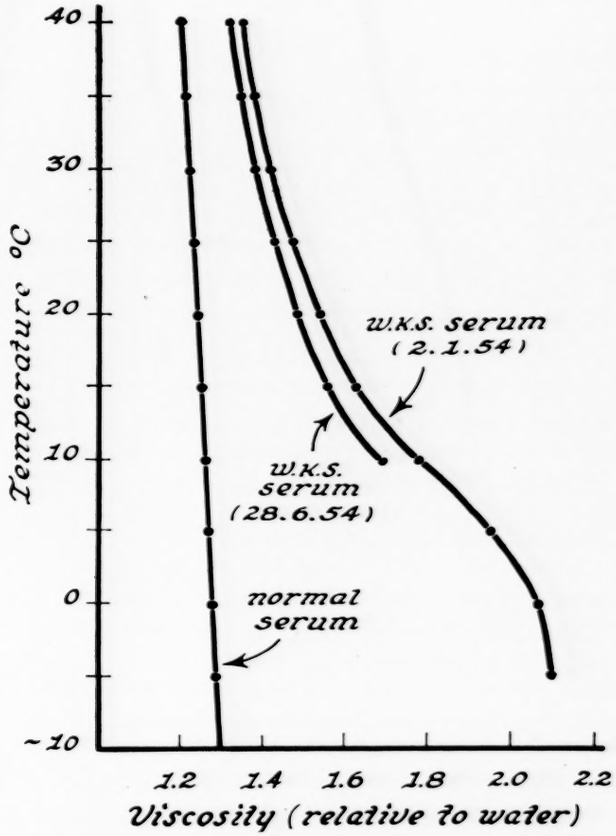


FIG. 13B. Patient W. K. S. Relative viscosity of cryoglobulin.

(1) When the undiluted serum was cooled to progressively lower temperatures there was an almost linear increase in the quantity of cryoglobulin precipitated. (Fig. 13A.) This increase was observed until freezing occurred. It is noteworthy that in some samples tested precipitation began at 28°C. and that at 23°C. (a temperature not uncommon in the toes) the precipitated cryoglobulin amounted to over 300 mg./100 cc.

(2) When a constant temperature (e.g., 2°C.) was maintained and aliquots of the serum diluted with isotonic saline solution (at 2°C.), the amount of precipitated protein greatly diminished, the remainder entering into solution. (Fig. 14.) When a 1:4 dilution was reached no cryoglobulin remained precipitated; all of the protein was dissolved. Normal serum and serum completely free from gamma globulin were utilized as diluents, as well as the patient's own supernatant serum (removed at −7°C. to render it unsaturated at 2°C.). These three sera gave equal results; with each, there was slightly more precipitation at the various serial dilutions when compared with the results of saline dilution. Nevertheless, no precipitate remained at the 1:4 dilution; and the curves were similar to those using normal saline solution.

(3) The temperature at which precipitation began was also greatly affected by changes in the concentration of the protein. (Fig. 15.) Serial dilution of the patient's serum with normal saline solution caused a marked fall in the temperature at which precipitation began. Similar results were likewise obtained using normal serum as a diluent.

These results emphasize the important fact that the cryoglobulin reaches a saturated state with only slight lowering of temperature, with consequent precipitation on further cooling. This marked reduction in solubility of the protein is in contrast with the much greater solubilities of other gamma globulins of similar or greater concentrations. The phenomenon of

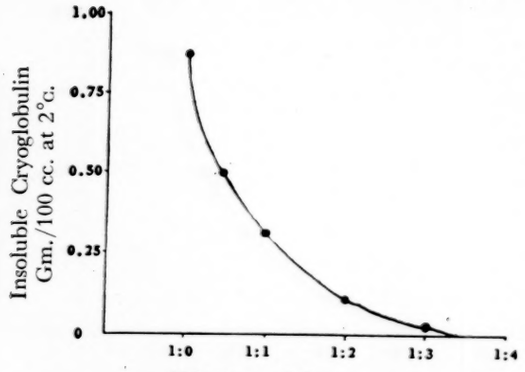


FIG. 14. Effect on the solubility of cryoglobulin when the patient's serum is diluted with physiologic saline (blood for this test drawn on May 8, 1954).

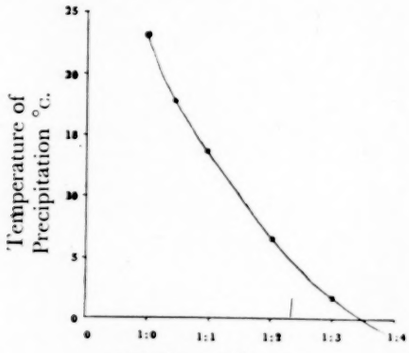


FIG. 15. Effect on the temperature of precipitation of cryoglobulin when the patient's serum is diluted with physiologic saline.

precipitation depends on the presence of a minimal amount of the protein (below which no precipitation ensues). The degree of precipitation varies directly with the concentration of the cryoglobulin and inversely with the temperature. An increasing concentration of cryoglobulin also leads to elevation of the temperature at which precipitation begins.

The *relative viscosity* of the cryoglobulin serum, normal serum and distilled water was estimated by timing the descent of each in a pipette similar to the capillary side of an Ostwald viscosimeter. There was a moderate increase in viscosity of the abnormal serum at body temperature and this was even more marked at lower temperatures. (Fig. 13B.)

Pertinent to the Nature of the Protein. Electrophoresis: Qualitative paper electrophoresis (Fig. 4) of the patient's serum was carried out in veronal buffer (pH about 8.6; ionic strength, 0.5) at room temperature. The cryoglobulin was found to migrate in the gamma globulin region. The zone of dispersion of this peak was less than half the usual, and this was similar to the abnormal gamma globulins obtained in some cases of multiple myeloma. There was also moderate increases in the alpha-1, alpha-2 and the beta fractions in the whole serum, but these fractions did not form a part of the cold-precipitable fraction.

These changes were confirmed by free electrophoresis (micro-Antweiler, barbital buffer, pH 8.5, ionic strength 0.1). In this case, however, (Fig. 5) in the whole serum a clear zone appeared between the beta and gamma peaks in the descending limb of the cell, representing actual precipitation in the cell. This precipitation was not a cold-precipitation, as the serum was diluted 1:15 in the buffer, at which dilution the cryoglobulin would not precipitate. The precipitation did not appear in the ascending boundary. It could thus represent a dissociation phenomenon, such as an unstable beta-lipoprotein complex. It would appear that under the conditions existing in the descending limb of the electrophoresis cell this dissociation had occurred; the lipid had precipitated out, leaving its associated protein in solution, travelling at approximately the same rate as fibrinogen.

When the supernatant fluid removed from the cooled specimen was similarly examined there was, as expected, much less gamma globulin than in the whole serum. In addition, no precipitation occurred in the descending limb of the cell. Therefore, although the cryoglobulin is a gamma globulin, there appears to be an associated beta-lipoprotein. The possible significance of this finding will be discussed subsequently.

By the sodium sulphite precipitation technic the cryoglobulin was found to be entirely in the euglobulin (15 per cent) fraction. By the sodium sulphate precipitation technic the cryoglobulin was found in the 8 to 17 per cent fractions, the maximum being in the 14 per cent fraction, no precipitate usually being obtained below 10 per cent.

Serologic tests: The cold-agglutinin titre was 1:8 (normal). ("Pseudo-agglutination" of red cells, due to enmeshing of the cells in the protein, occurred before dilution.) The presumptive Kahn and direct Coombs tests were negative.

The anticomplementary titre of the serum was 1:512. This remarkably high titre was found to be resident entirely in the cryoglobulin fraction.

Effect of heating serum at 56°C.: When the serum was heated at 56°C. for thirty minutes the property of cold-precipitability of the protein was lost completely, or almost completely. This property did not reappear even when the serum was subsequently refrigerated for long periods. The addition of varying concentrations of guinea pig complement failed to result in a return of the ability of the protein to precipitate in the cold.

The effect of heating in this manner on the electrophoretic pattern was of interest. There was no significant change in the amount of gamma globulin (although more prolonged heating—ninety minutes—caused a moderate decrease). The alpha-1 fraction disappeared in the normal sera as well as the patient's serum but this occurred more rapidly in the latter. The mobility of the alpha-2 fraction was decreased, producing a merging of the alpha-2 and beta fractions. A slight increase in the beta fraction was also observed. The albumin fraction remained unchanged.

These changes in the alpha-2 and beta fractions were not observed in two normal sera similarly studied.

Effect of repeated rewarming of serum to 37°C.: The serum was repeatedly cooled to 3°C. and then rewarmed to 37°C. As this sequence was repeated the amount of cold-precipitable protein diminished. There was very considerable variation from sample to sample; however, by the time this procedure had been carried out five to fifteen times in rapid succession the property of cold-precipitation of the protein was permanently lost. A gradual decrease in cold precipitability was noted after prolonged standing at room temperature, and this process was slightly accelerated by incubation of the serum at 37°C. for lengthy (e.g., three week) periods.

Effect of refrigerating serum for prolonged periods: The consistency of the cryoglobulin in samples of serum refrigerated continuously for one week or longer was found to increase, forming a much firmer gel than had been noted in fresh specimens. The extent of this change varied directly with the length of time in the refrigerator. In addition, the protein became increasingly insoluble after prolonged refrigeration; after several weeks at 3°C. only part (e.g., 70 to 80 per cent) of the cryoglobulin could be redissolved by warming to 37°C. for forty-eight hours, or with continuous agitation, six to eight hours.

Addition of heparin: The patient's heparinized plasma and serum to which heparin was added showed certain differences in behaviour from the untreated serum. Following initial precipitation in the refrigerator, and without removing the sample from the refrigerator, the amount of precipitated cryoglobulin (in the heparinized plasma, and also to a lesser extent in the serum plus heparin) became less and less, until after two weeks, none remained precipitated; no further precipitate appeared, even when cooled further to −7°C.

The heparinized plasma, heparinized serum and unheparinized serum were incubated at 37°C. for two weeks. When these specimens were then cooled to −7°C. there was significantly less precipitation in the heparinized samples than in the unheparinized serum.

Heparin appeared to facilitate the electrophoretic merging of alpha-2 with beta globulin but would not accomplish these changes without heating to 56°C. The more marked merging of the alpha-2 and beta fractions was accompanied by a greater increase in the beta fractions than was noted when heparin was not added. These changes were not observed in two normal sera similarly studied.

Ultracentrifugation: The cryoglobulin was washed with distilled water and tested apart from the remainder of the serum. It was found to have a sedimentation constant identical with that of normal gamma globulin (in the S3 range). The molecular weight was estimated to be 167,000.

COMMENTS

Aetiology. The cause of this patient's cryoglobulinaemia remains unknown. The presence of cryoglobulin is usually a secondary phenomenon often but not necessarily related to hypergammaglobulinaemia. Its presence "may be considered a non-specific indication of a disease process comparable to fever, elevated erythrocyte sedimentation rate, etc."[11] However, in the case herein reported, extensive clinical and necropsy investigation failed to reveal any underlying disease entity (aside from chronic bronchitis and emphysema). The twenty-five-year history, coupled with this failure to demonstrate an underlying cause, places this case in the category of "essential cryoglobulinaemia." Whether or not the presence of cryo-

globulinaemia represented an immune response to an unknown antigen is a matter for speculation.

Because the patient's manifestations occurred only after exposure to cold until nine months before death, the question of "cold allergy" has to be considered. Only three cases of true "cold allergy" appear in the literature,[47–49] two resembling our case closely. However, examination of the sera for cryoglobulin was not made in any of them. It is our belief that patients with cryoglobulinaemia are *not* primarily allergic to cold. The property of cold precipitation is a physical one, related to the decreased solubility of the protein, which therefore precipitates when cooled.

Although cold agglutinins may rarely cause clinical manifestations similar to those due to cryoglobulinaemia,[50–51] the coexistence of the two has been reported only once.[29] In our case, as in others,[12,14,32–34] the titre of cold agglutinins was normal. It is true that a pseudo-agglutination of red cells occurs when the patient's serum is cooled but this consists merely of an enmeshing of erythrocytes in the precipitated protein.[52] When the serum was diluted fourfold the cryoglobulin ceased to be precipitable and this pseudo-agglutination was not observed. However, it is interesting to note that in the case of Schwartz and Jager,[29] in which a cold-precipitable protein and a cold agglutinin were coexistent, it was found that the property of cold agglutination was resident in the cryoglobulin; even when a very dilute solution of this protein in saline was tested, agglutination titres were very high.

Our observations have established that dilution of the patient's serum with his own supernatant serum, normal serum or normal saline solution decreased its precipitability by cold, and that diluting the serum to 1:4 caused all the cryoglobulin to remain in solution at 2°C. A very small amount of this identical protein could therefore be present in the serum and not be precipitated by cold. In preliminary work by Reader and De Gara[53] with precipitin tests, negative results were obtained when the sera of normal subjects were cross matched with anti-cryoglobulin. This suggests that cryoglobulin is not a normal constituent of blood even in small quantity. However, Morrison et al.[54] recovered a "cold-insoluble" globulin in pooled "normal" human plasma. This material constituted 0.3 to 0.7 per cent of the total plasma protein. These investigators considered that this was not a normal blood protein but was contributed to the pooled plasma by a small percentage of donors. No proof in support of this theory was offered.

The question arises why this protein should have such a low solubility when frequently much greater concentrations of gamma globulin have no such characteristic. Our studies suggest that there may be an immunologic process affecting some or all of the gamma globulin and probably requiring the presence of complement. This possibility is suggested by the following factors: (1) The high anticomplementary titre (of 1:512) which was resident in the cryoglobulin. Davis et al.[77] have advanced evidence that such a titre indicates complement fixation by the gamma globulin. (2) Heating the serum to 56°C. for thirty minutes caused the property of cold-precipitation to become greatly reduced or disappear altogether. This suggested that a heat-labile substance was inactivated at this temperature or, alternately, that the heating caused dissociation of a protein complex. (3) The inhibitory effect of heparin on the precipitation of the protein. (Heparin has been shown to exert an antagonistic effect on the action of complement.[55]) (4) The presence of an unstable beta lipoprotein in association with the cryoglobulin (in the free electrophoresis patterns) suggested an association between the gamma globulin and the unstable beta-lipoprotein.

It might be pertinent to recall that whole complement is inactivated by heating to 56°C. Further, the C′1 (mid-piece) fraction of complement has been found to be a beta globulin,[56,57] while the C′3 fraction is believed to be a phospholipid.[58] (C′2 or end piece is a muco-alpha-globulin, and C′4 is also an alpha globulin.[56,57]) Since the total concentration of the protein moieties of complement is in the order of 40 mg. per 100 cc.,[59] the fractions of complement are too small to be evident in routine electrophoretic patterns. Nevertheless, an attractive speculation is that the unstable beta-lipoprotein-cryoglobulin complex might represent an immunologic association; the complement may also be mirrored in these changes.

The merging of the alpha-2 and beta globulin fractions in the electrophoresis patterns with heating, and the enhancing effect on this electrophoretic change with the addition of heparin, are effects which are as yet unclear.

When the serum was heated to 56°C. for thirty minutes, and then varying concentrations of guinea pig complement added, the property

of cold-precipitation did not reappear. However, it has been shown that fractions of guinea pig complement are not completely interchangeable with human complement,[61] and it may be that this species difference was a limiting factor. Although the precise nature of the association phenomenon is not clear, it seems evident that an immunologic relation with the gamma globulin is essential for the production of the state of decreased solubility.

Although the most commonly occurring cryoglobulins have occurred in the gamma fraction, this is by no means a universal finding. A number of other proteins have been found which exhibit this property of cold-precipitation. These include globulins in the alpha fraction,[16] beta fraction,[8,25,28,62,81] and between beta and gamma peaks.[21,31] Numerous physicochemical studies have been carried out on these cryoglobulins;[8–15,17–19,28,60,62–66] although considerable variation has been noted in molecular weights (165,000 to 600,000) and in some of the physical properties and chemical constituents, the characteristic of cold insolubility is the common denominator. Thus it may be concluded that there is a group of different proteins which display this anomalous physical characteristic. (Another group of proteins with a common anomalous physical characteristic is Bence-Jones protein.[67]) A single protein with a further unusual physical characteristic is the "pyroglobulin" of "pyroglobulinaemia."[68]

The source of this protein in our patient is unknown. Unlike most patients manifesting large quantities of cryoglobulin, there was no increase in plasma cells in the bone marrow. In the group of cases in which an increased number of plasma cells were encountered, it is certainly possible that plasma cells were the site of production of cryoglobulin, as Barr et al.[12] have suggested. There are now reported a small number of cases of cryoglobulinaemia similar to our own in that plasma cells have not been present in increased numbers in the bone marrow.[8,10,28,29,31] It can only be concluded that plasma cells are by no means the invariable source of this protein. The route of disposal also is not known but it seems conceivable from the demonstration of inclusions of precipitated cryoglobulin in leukocytes *in vitro*[41] that the cryoglobulin which is precipitated *in vivo* may be removed at least in part by phagocytosis.

Pathogenesis and Pathology. The manifestations of cryoglobulinaemia (whatever the underlying disease) have heretofore been ascribed to the simple precipitation of the abnormal protein in blood vessels *in vivo*. While this precipitation remains the undoubted basic mechanism in the production of the lesions, there is certain evidence that in our patient, at least, another factor is involved. It would appear that a tissue reaction to the protein was essential for the appearance of the manifestations, i.e., the cryoglobulin, as it precipitated (as a result of concentration, temperature and viscosity), acted as a foreign protein introduced into the body with the expected subsequent local and systemic reactions. This hypothesis assumes a sensitivity to the protein when precipitated that is absent when it is in solution. Such a state could be due to a qualitative change in the protein or an increase in its local concentration on precipitation.

This interpretation followed the observation that long-term ACTH therapy prevented the recurrence of purpura and the other lesions, although the concentration and precipitability of the cryoglobulin remained virtually unchanged. Presumably, the cryoglobulin was continuing to precipitate intravascularly as before but due to the lack of local tissue reaction there was no actual thrombus formation and therefore no purpura. The rigors and fever experienced by the patient twenty-four hours prior to each bout of purpura suggests also a systemic reaction to the precipitating protein. (Early in the illness this sequence occurred only after exposure to cold, although later it occurred while the patient was under bedcovers.) The latter manifestations were similarly inhibited by the ACTH therapy; thus it would appear that the hormone repressed both the local and systemic reactions to the precipitating protein, without modifying the probable intravascular precipitability. Steinhardt and Fischer[32] have considered that the wheal produced by placing an ice cube on the skin of a patient with cryoglobulinaemia constitutes evidence of an allergic factor in this condition and have shown that while antihistaminics reduce this wheal, ACTH had no effect on this phenomenon. We have repeated these observations and our own experience is similar. However, this phenomenon may be due to a local tissue reaction to a "foreign" protein (the locally precipitating cryoglobulin) rather than actual hypersensitivity.

It is therefore considered that the lesions are a result of local precipitation and a local reaction to the precipitating protein. This may be

reversible or irreversible, the latter resulting in death of tissue. Raynaud's phenomenon has been frequently described in this condition, and is an example of the former. Our clinical observations in this regard are similar to those of Hansen and Faber,[13] and suggest histamine release when the hand is warmed after cooling. Schwartz and Jager[29] have established an element of arteriospasm in their case but this element did not appear to be operative in our patient.

With the use of a biomicroscope Barr et al.[12] have noted aggregation of erythrocytes and slowing of the circulation in the minute vessels of the conjunctiva in a patient with cryoglobulinaemia. Following application of a tube of ice water to the eyeball the "sludging" of blood became excessive, with segmentation of columns of blood and, in some cases, reversal of blood flow. These changes were evident for several minutes following withdrawal of the cold stimulus. These findings are probably analogous to those causing the atypical Raynaud's phenomenon, and indicate that a transient precipitation of the protein with temporary occlusion of blood flow in small blood vessels is the likely explanation for the Raynaud's phenomenon. The tender, ulcerative, haemorrhagic, slowly healing skin lesions are undoubtedly due to necrosis of small areas of skin (unlike simple purpura which has none of these characteristics). It is suggested that occlusion of the small blood vessels of the skin initiated by the cryoglobulin results in ischemia, with subsequent necrosis. The haemorrhagic stomatitis, epistaxis, retinal haemorrhages and petechiae in the intestinal tract may be similarly explained. Recently, cerebral purpura has been described,[23] and a similar explanation may be invoked. The gangrene may be explained by the deposition of cryoglobulin in small arteries with attendant thrombosis and occlusion. Organization with some recanalization may occur.[34] Dyspnoea and cyanosis may be due to occlusion of the pulmonary arterial tree[37] with locally precipitated cryoglobulin or pulmonary emboli from venous thrombosis. The deafness could be caused by gelation of the endolymph, although this is speculative. Coagulated cryoglobulin has been reported to be present in the heart and great vessels,[69] pulmonary arterioles,[37] multiple arteries and veins,[16] and most of the small visceral vessels,[12,13] as well as extremities.[13,16,34–36] The cause of death is often due directly to the underlying disease but may be caused by the precipitation of this protein, and subsequent ischemia, of vital areas.[1,12,13,16]

The interesting question arises as to why precipitation of protein would occur in areas of the body where the temperature is not appreciably affected by the external environment. One possible explanation lies in the evidence that increasing the concentration of cryoglobulin leads to progressively higher and higher temperatures of precipitation. (Fig. 15.) Thus, if a patient is developing a greater concentration of cryoglobulin it may be expected that his manifestations will occur at warmer temperatures. This was observed in the clinical course of our patient, whose signs and symptoms originally appeared only on exposure to cold but which later occurred even at room temperatures. Ultimately saturation may become so great that precipitation at 37°C. will occur.[12]

The viscosity of the blood even at 37°C. in many reported cases has been increased, thus adding an additional factor leading to thromboses in the circulating blood stream.

Clinical Manifestations. Of the great number of patients with cryoglobulinaemia, very few have manifestations attributable to this abnormal protein. With one recorded exception,[12] large concentrations of cryoglobulin appear to be necessary in order to produce signs and symptoms. (However, MacKay et al.[83] mention two patients with massive concentrations of cryoglobulin without resultant clinical manifestations.) It is further suggested that proteins which take more than twenty-four hours to precipitate in the cold (*in vitro*) are very unlikely to be of immediate clinical importance. Cold sensitivity, Raynaud's phenomenon, epistaxis, stomatitis, deafness, retinal haemorrhages, purpura, gangrene, blotchy pigmentation, venous thromboses, pulmonary infarcts, melaena, cerebral purpura, dyspnoea and cyanosis are well known manifestations of cryoglobulinaemia, no matter what the underlying cause. Although these signs and symptoms might suggest a "collagen disease," sufficient postmortem evidence (from many reported cases[1,12–16,29] as well as our own) is now at hand to refute such a supposition, although collagen diseases are among the known causes of cryoglobulinaemia.

To the list of findings cited it would appear that rigors and fever must be added. It may be noted that, over the years, the episodes of purpura in our patient occurred invariably about twenty-four hours after a true rigor. It

has been speculated that a tissue reaction to the precipitating protein is essential in order for the manifestations to make their appearance.

The clinical course depends frequently upon the underlying disease. However, in the group of patients with "essential cryoglobulinaemia" the history may extend over many years. An increasing concentration of cryoglobulin leads to increasingly severe disturbances. Nevertheless, the clinical course may be marked with remissions and exacerbations (often seasonal), particularly in the early years. Just prior to ACTH therapy our patient demonstrated evidence that he was finally approaching an end stage of his disease and, indeed, appeared terminal on more than one occasion. It may be that when a patient has accumulated sufficient cryoglobulin to exhibit manifestations at room temperature or higher, the disease becomes unremittingly progressive.

Two other dysproteinaemias produce similar clinical manifestations, i.e., macroglobulinaemia[71–72] and benign hyperglobulinaemic purpura.[73–75] In the former case the entire serum gels at room temperature, but there is no actual precipitate. In the latter condition there is neither a gel nor a precipitate in the cooled serum. Macroglobulinaemia and cryoglobulinaemia have, on occasion, been noted in the same patient.[83]

Response to ACTH and Cortisone. In most cases of cryoglobulinaemia treatment is directed toward the underlying disease. In our case, since a known cause was lacking, treatment was directed toward a "manifestation," i.e., the derangement in plasma proteins. When it was learned that this cryoglobulin was a gamma globulin it was considered that ACTH or cortisone might be of value because of their known effect in decreasing gamma globulin (and euglobulin) in other conditions.[76,82] It was on this basis that hormone therapy was instituted.

Short-term studies using ACTH have been recorded on three previous occasions. Engle and Barr[38] reported its use in a case of multiple myeloma with cryoglobulinaemia. The drug was administered for two periods of ten and a half and seven days, respectively, but there was no effect on serum proteins or symptoms. In the case of essential cryoglobulinaemia discussed by Steinhardt and Fischer,[32] ACTH and cortisone caused a decrease in purpuric manifestations and a decrease in cryoglobulin but treatment was carried on for only eight days. Aside from purpura, other signs and symptoms were unaffected. In Pelzig's case,[33] ACTH caused a marked decrease in the cryoglobulin, but had little clinical effect.

In a study of a case of lymphocyte leukaemia with autoimmune haemolytic disease and cryoglobulinaemia, Craig et al.[80] utilized ACTH and cortisone for periods up to twenty-five days. However, there was no mention of symptomatology directly referable to the cryoglobulin, and no studies of the effect of the therapy on the serum proteins were presented.

There can be little doubt from our study that the ACTH exerted a highly beneficial clinical effect for a prolonged albeit temporary period. The establishment of therapy (on December 11, 1953, and January 7, 1954), and in one instance the change from cortisone to ACTH, converted a patient who appeared terminal on those occasions to one relatively asymptomatic. It was soon found that administration of the hormone could not be discontinued for more than forty-eight hours without return of fever and the like. Until the last month of life ACTH prevented appearance of major manifestations of the disease.

Despite the prolonged beneficial effect of ACTH there was little change in the continual high concentration of the cryoglobulin. However, it should be noted that the serum level of gamma globulin was definitely decreased by long-term ACTH therapy. Indeed, a marked decrease in gamma globulin was discovered within one day following institution of therapy. When the ACTH was temporarily discontinued in early January the patient's gamma globulin increased rapidly within a few days. After March 1st the gamma globulin gradually decreased, even when determined just prior to death.

Toward the end of the study the cryoglobulin came to compose most of the gamma globulin; and when it is considered that only the oversaturated cryoglobulin precipitates, the remainder staying in solution, even more of the gamma globulin than is shown as cryoglobulin consists of the abnormal protein.

Although the euglobulin concentration (sodium sulphite technic) reacted more slowly to ACTH, it also showed a definite decrease with long-term therapy. The cryoglobulin came to compose most of this fraction by this salting-out technic, as only the non-cryoglobulin part of the euglobulin appeared to respond to ACTH

therapy. The failure of the cryoglobulin to show quantitative changes with ACTH is difficult to explain but might be due to inability of the hormone to affect the cells producing the abnormal protein, or to an abnormally long half-life of the cryoglobulin, or perhaps to some qualitative feature of the protein itself.

To explain the remarkable effect of ACTH on the clinical course, therefore, one cannot invoke significant quantitative alterations in cryoglobulin. An appealing theory is that the ACTH exerts an anti-inflammatory effect which is non-specific. Since sensitivity, perhaps to the patient's own precipitating cryoglobulin, may have been in great part responsible for the appearance of the lesions described, it may be further suggested that the ACTH prevented this response and thus alleviated symptomatology.

SUMMARY

A case of cryoglobulinaemia is presented in which extensive investigation and postmortem examination failed to reveal an underlying cause. The clinical manifestations included an unusually long history of cold sensitivity and purpura, rigors, fever, atypical Raynaud's phenomenon, deafness, epistaxis, stomatitis, postnasal bleeding, dyspnoea, cyanosis, pulmonary emboli, melaena, blotchy pigmentation of lower extremities, thrombophlebitis and gangrene of toes. This patient did not exhibit retinal haemorrhages.

Cryoglobulin may be said to be the generic term for a group of serum proteins which have the common physical property of precipitating on exposure to the cold. This precipitation is due to a state of decreased solubility of the protein in the blood, and has been demonstrated to occur *in vitro* at temperatures occurring within the body. The possible mechanisms of production of this relative insolubility and the possible role of complement and lipoprotein are discussed.

Cryoglobulinaemia is most often a secondary phenomenon. This protein may be found in small quantities in many disease states but large concentrations are rare. When large amounts are found multiple myeloma is frequently the cause. Occasionally, as in our case, no definite aetiology is demonstrated.

It is suggested that tissue reaction is a factor in the pathogenesis of the lesions; and the possibility exists that the patient's own precipitating cryoglobulin might have acted as a foreign protein, stimulating a tissue response with associated rigors and fever, and resulting in subsequent purpura.

The theory that this type of reaction is of great importance is further supported by the favourable results of treatment with ACTH, without significant decrease in the concentration of cryoglobulin.

The pathologic condition of this disorder appears to depend upon the initial deposition of cryoglobulin in blood vessels of varying size, with subsequent local reaction, secondary thrombosis, ischemia of the tissue supplied by the vessels and resultant necrosis. An increasing concentration of cryoglobulin may lead to the appearance of lesions at higher and higher temperatures, and may explain the appearance of lesions in areas of the body where the temperature could not be materially affected by the external environment.

ADDENDUM

Since this article was submitted, two additional cases of "essential" cryoglobulinaemia have come to our attention. F. H. Gardner of Boston (personal communication) has treated, with ACTH, a middle-aged woman with large concentrations of cryoglobulin and moderately severe haemolytic anaemia. The cryoglobulin disappeared, the haemolytic episode subsided concomitant with the ACTH therapy, and the patient has since been well. F. W. Gunz of New Zealand (personal communication) has described the case of a forty-four year old woman with a thirteen-year history of cold-induced urticaria and purpura but no visceral manifestations. The cryoglobulin amounted to about 1.1 gm./100 cc. This patient has run a fairly benign course, and no steroid therapy has been given.

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REFERENCES

1. Wintrobe, M. M. and Buell, M. V. Hyperproteinemia associated with multiple myeloma: with report of a case in which an extraordinary hyperproteinemia was associated with thrombosis of the retinal veins and symptoms suggesting Raynaud's disease. *Bull. Johns Hopkins Hosp.*, 52: 156, 1933.
2. Von Bonsdorff, B., Groth, H. and Packalen, T. On the presence of a high molecular crystallizable protein in blood serum in myeloma. *Folia haemat.*, 59: 184, 1938.
3. Bing, J. Further investigations on hyperglobulinemia. I. Occurrence and degree of hyperglobulinemia in the various diseases; the ratio between hyperglobulinemia, hyperproteinemia, and hypoalbuminemia; the formol gel reaction. *Acta med. Scandinav.*, 103: 547, 1940.
4. Stein, L. and Wertheimer, E. A new fraction of cold-susceptible protein in dogs infected with kala-azar. *Ann. Trop. Med.*, 36: 17, 1942.
5. Holmberg, C. G. and Gronwall, E. Ein neues krystalinisches serum Globulin. *Ztschr. f. physiol. Chem.*, 273: 199, 1942.
6. Wertheimer, E. and Stein, L. The cold susceptible globulin fraction of pathologic sera. *J. Lab. & Clin. Med.*, 29: 1082, 1944.
7. Flemberg, T. and Lehmann, J. Discussion of paper by Waldenström, J. *Nord. med.*, 23: 1565, 1944.
8. Waldenström, J. Incipient myelomatosis or "essential" hyperglobulinemia with fibrinogenopenia—a new syndrome? *Acta med. Scandinav.*, 117: 216, 1944.
9. Shapiro, R. and Wertheimer, E. Spontaneous crystallization of a protein from pathological human serum. *Brit. J. Exper. Path.*, 27: 225, 1946.
10. Lerner, A. B. and Watson, C. J. Unusual purpura associated with the presence of a high concentration of cryoglobulin (cold precipitable serum globulin). *Am. J. M. Sc.*, 214: 410, 1947.
11. Lerner, A. B., Barnum, C. P. and Watson, C. J. Studies of cryoglobulins. II. The spontaneous precipitation of protein from serum at 5°C. in various states. *Am. J. M. Sc.*, 214: 416, 1947.
12. Barr, D. P., Reader, G. G. and Wheeler, C. H. Cryoglobulinemia. I. Report of two cases with discussion of clinical manifestations, incidence and significance. *Ann. Int. Med.*, 32: 6, 1950.
13. Hansen, P. F. and Faber, M. Raynaud's syndrome originating from reversible precipitation of protein. *Acta med. Scandinav.*, 129: 81, 1947.
14. Hill, R. H., Dunlop, S. G. and Mulligan, R. M. A cryoglobulin present in high concentration in the plasma of a case of multiple myeloma. *J. Lab. & Clin. Med.*, 34: 1057, 1949.
15. Rorvik, K. Cryoglobulinemia: a survey and a case report. *Acta med. Scandinav.*, 137: 390, 1950.
16. Cugudda, E. Plasmacitoma con cryoglobulinemia e trombosi arteriose e venose multiple. *Minerva med.*, 43: 205, 1952.
17. Putnam, F. W. and Udin, B. Proteins in multiple myeloma. I. Physico-chemical study of serum proteins. *J. Biol. Chem.*, 202: 727, 1953.
18. Dustin, J. P. Isolément d'une cryoglobuline dans un sang myelomateux. *Arch. internat. de physiol.*, 61: 256, 1953.
19. Brauman, J., Gregoire, F., Lambert, P. P., Kleyntjens, F. and Danis, P. Un cas de myeloma à cryoglobulin: Étude clinique, chimique, et physiologique. *Acta clin. belg.*, 8: 333, 1953.
20. Blades, A. N. Cryoglobulinaemia in multiple myelomatosis. *Brit. M. J.*, 1: 169, 1951.
21. McFarlane, A. S. and Dovey, A. An unusual case of hyperglobulinaemia. *J. Path. Bact.*, 64: 335, 1952.
22. Cosgrove, K. E. and La Tourette, K. A. Multiple myeloma simulating hyperparathyroidism. *Am. J. Med.*, 15: 863, 1953.
23. Marshall, R. J. and Malone, R. G. S. Cryoglobulinaemia with cerebral purpura. *Brit. M. J.*, 2: 279, 1954.
24. Most, H. and Lavietes, P. H. Kala-azar in American military personnel. *Medicine*, 26: 221, 1947.
25. Lepow, H., Rubenstein, L., Woll, F. and Greisman, H. A spontaneously precipitable protein in human sera, with particular reference to the diagnosis of polyarteritis nodosa. *Am. J. Med.*, 7: 310, 1949.
26. Dreyfuss, F. and Librach, G. Cold precipitable serum globulins ("cold fractions," "cryoglobulins") in subacute bacterial endocarditis. *J. Lab. & Clin. Med.*, 40: 489, 1952.
27. James, T. N. and Drake, E. H. Cryoglobulins in coronary artery disease. *New England J. Med.*, 249: 601, 1953.
28. Abrams, A., Cohen, P. P. and Meyer, O. O. The physical properties of a cryoglobulin obtained from lymph nodes and serum of a case of lymphosarcoma. *J. Biol. Chem.*, 181: 237, 1949.
29. Schwartz, T. B. and Jager, B. V. Cryoglobulinemia and Raynaud's syndrome in a case of chronic lymphocytic leukemia. *Cancer*, 2: 319, 1949.
30. Israels, L. and Kilgore, J. Cryoglobulinaemia in polycythaemia rubra vera. *Proc. Roy. Coll. Phys. Canad.*, 1954.
31. Griffiths, L. L. and Gilchrist, L. Cryoglobulinaemia in alcoholic cirrhosis. *Lancet*, 264: 882, 1953.
32. Steinhardt, M. J. and Fischer, G. S. Cold urticaria and purpura as allergic aspects of cryoglobulinemia. *J. Allergy*, 24: 335, 1953.
33. Pelzig, A. Essential cryoglobulinemia with purpura. *Arch. Dermat. & Syph.*, 67: 429, 1953.
34. Hutchinson, J. H. and Howell, R. A. Cryoglobulinemia: report of a case associated with gangrene of the digits. *Ann. Int. Med.*, 39: 350, 1953.
35. Wright, I. S. The Pathogenesis and Treatment of Thrombosis, p. 20. New York, 1952. Grune & Stratton.

36. Butler, K. R. and Palmer, J. A. Cryoglobulinaemia in polyarteritis nodosa. *Canad. M. A. J.*, 72: 686, 1955.
37. Muirhead, E. E., Montgomery, P. O. and Gordon, C. E. Thromboembolic pulmonary vascular sclerosis: report of a case following pregnancy, and of a case associated with cryoglobulinemia. *Arch. Int. Med.*, 89: 41, 1952.
38. Engle, R. L. and Barr, D. P. Multiple Myeloma Treated With ACTH. Proc. 2nd Clin. ACTH Conf. (Therapeutics). vol. 2, p. 209. Edited by Mote, J. R. New York, 1951. Blakiston Co.
39. Campbell, W. R. and Hanna, M. I. The albumin, globulin and fibrinogen of serum and plasma. *J. Biol. Chem.*, 15: 119, 1937.
40. Winzler, R. J., Devor, A. W., Mehl, J. W. and Smyth, I. W. Studies on the mucoproteins of human plasma. I. Determination and isolation. *J. Clin. Investigation*, 27: 609; 1948.
41. Volpé, R. and Ogryzlo, M. A. The cryoglobulin inclusion cell. *Blood*, 10: 493, 1955.
42. Gornall, A. G., Bardawill, C. J. and David, M. M. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.*, 177: 751, 1949.
43. Majoor, C. L. H. The possibility of detecting individual proteins in blood serum by differentiation of solubility curves in concentrated sodium sulfate solutions. *Yale J. Biol. & Med.*, 18: 419, 1946.
44. Cullen, G. E. and Van Slyke, D. D. Determination of the fibrin, globulin, and albumin nitrogen of blood plasma. *J. Biol. Chem.*, 41: 487, 1920.
45. Folin, O. and Wu, H. A system of blood analysis supp. I: a simplified and improved method for determination of sugar. *J. Biol. Chem.*, 41: 367, 1920.
46. M. R. C. Committee on Clinical Endocrinology. Proposed standard method of 17-ketosteroid determination. *Lancet*, 261: 585, 1951.
47. Sack, G. Beitrag zur vascularen Purpura und ein neuer Vorschlag zur Behandlung. *Deutsch. Arch. f. klin. Med.*, 185: 186, 1939.
48. Peters, G. A. and Horton, B. T. Allergic purpura with special reference to hypersensitiveness to cold. *Proc. Staff Meet., Mayo Clin.*, 16: 631, 1941.
49. Urbach, E., Herman, M. F. and Gottlieb, P. M. Cold allergy and cold pathergy. *Arch. Dermat. & Syph.*, 43: 366, 1941.
50. Stats, D. and Wassermann, L. R. Cold hemagglutination—an interpretive review. *Medicine*, 22: 363, 1943.
51. Ferriman, D. G., Dacie, J. V., Keele, K. D. and Fullerton, J. M. The association of Raynaud's phenomena, chronic haemolytic anaemia and the formation of cold antibodies. *Quart. J. Med.*, 20: 275, 1951.
52. Luckey, E H., Russ, E. and Barr, D. P. Cryoglobulinemia. II. Influence of a cryoglobulin on the suspension stability and sedimentation rate of erythrocytes. *J. Lab. & Clin. Med.*, 37: 253, 1951.
53. Reader, C. G. and De Gara, P. F. Unpublished data. Cited by Barr, D. P., Reader, C. G. and Wheeler, C. H.[12]
54. Morrison, P. R., Edsall, J. T. and Miller, S. G. The separation of purified fibrinogen from human plasma fraction. *J. Am. Chem. Soc.*, 70: 3103, 1948.
55. Ecker, E. E. and Pillemer, L. Anti-coagulants and complementary activity. An experimental study. *J. Immunol.*, 40: 73, 1941.
56. Pillemer, L., Ecker, E. E., Oncley, J. L. and Cohn, E. J. The preparation and physicochemical characterization of the serum protein components of complement. *J. Exper. Med.*, 74: 297, 1941.
57. Ecker, E. E. and Pillemer, L. Complement. *Ann New York Acad. Sc.*, 43: 63, 1942.
58. Ecker, E. E., Jones, C. B. and Kuehn, A. O. Studies on the adsorption of complement. *J. Immunol.*, 40: 81, 1941.
59. Heidelberger, M. Complement: immunity intensifier, diagnostic drudge, chemical curiosity. *Am. Scientist*, 34: 597, 1946.
60. Pederson, K. O. Size relationships among similar proteins: association and dissociation reactions of protein units. *Cold Spring Harbor Symp., Quant. Biol.*, 14: 140, 1950.
61. Ecker, E. E., Pillemer, L. and Seifter, S. Immunochemical studies on human serum I. Human complement and its components. *J. Immunol.*, 47: 181, 1943.
62. Lucey, H. C., Leigh, E., Hock, H., Marrack, J. R. and Johns, R. G. S. Study of a case of purpura associated with bone changes and formation of a gel in the serum on standing. *Brit. J. Exper. Path.*, 31: 380, 1950.
63. Lerner, A. B. and Greenberg, G. R. A homomolecular serum protein with anomalous solubilities. *J. Biol. Chem.*, 162: 428, 1946.
64. Harvier, P., Turiaf, J. and Delbarre, F. Purpura hemorrhagique, syndrome de Raynaud, et cryoproteinémie. *Presse méd.*, 61: 1549, 1953.
65. Dustin, J. P. and Leonis, J. Etude chimique d'une cryoglobuline isolée. *Arch. internat. de physiol.*, 61: 422, 1953.
66. Hardy, S. and Putnam, F. W. Proteins in multiple myeloma. IV. Interaction with metabolic nitrogen. *J. Biol. Chem.*, 212: 371, 1955.
67. Gutman, A. B. The plasma proteins in disease. In: Advances in Protein Chemistry, vol. 4, p. 201. Edited by Anson, M. L. and Edsall, J. T. New York, 1948. Academic Press.
68. Martin, W. J. and Mathieson, D. R. Pyroglobulinemia (heat coagulable globulin in the blood). *Proc. Staff Meet., Mayo Clin.*, 28: 545, 1953.
69. Propp, S., Gorham, L. W. and Kantor, S. Recent studies of multiple myeloma: sternal and rib puncture and the results of treatment with stilbamide. *Blood*, 4: 36, 1949.
70. Bianchi, V., Giampalmo, A. and Marmont, A. Contributo alla conoscenza della gelificazione plasmatica a frigore e della plasmocitosi aleucemica con emogelificazione (gel-plasmocitomatosi). *Minerva med.*, 2: 101, 1949.
71. Tischendorf, W. and Hartmann, F. Makroglobulinaemie (Waldenström) mit gleichzeitiger Hyperplasie der Gewebsmastzellen. *Acta haemat.*, 4: 374, 1950.
72. Wilde, H. and Hitzelberger, A. L. Macroglobulinemia, clinical features and differential diagnosis. *Blood*, 9: 875, 1954.
73. Waldenström, J. Clinical methods for determination of hyperproteinemia and their practical

value for diagnosis: contributing to study of "essential high sedimentation reaction." *Nord. med.*, 20: 2288, 1943.

74. Waldenström, J. Three new cases of purpura hyperglobulinemica. A study in longlasting benign increase in serum globulin. *Acta med. Scandinav.* (suppl.), 226: 931, 1952.
75. Taylor, F. E. and Battle, J. D. Benign hyperglobulinemic purpura: case report. *Ann. Int. Med.*, 40: 350, 1954.
76. Jager, B. V., Brown, H. and Nickerson, M. Alterations in plasma proteins, plasma volume, and volume of packed red cells in patients receiving ACTH or cortisone. *J. Lab. & Clin. Med.*, 37: 431, 1951.
77. Davis, B. D., Kabat, E. A., Harris, A. and Moore, D. H. The anticomplementary effect of serum gamma globulin. *J. Immunol.*, 49: 223, 1944.
78. Asserman, E. F. and Hines, M. S. Multiple myeloma with cryoglobulinemia treated with 6-mercaptopurine. *Cancer Res.*, 1: 36, 1954.
79. Osserman, E. F. and Lawlor, D. P. Abnormal serum and urine proteins in thirty-five cases of multiple myeloma as studied by filter paper electrophoresis. *Am. J. Med.*, 18: 462, 1955.
80. Craig, A. B., Waterhouse, C. and Young, L. E. Auto-immune hemolytic disease and cryoglobulinemia associated with chronic lymphatic leukemia. *Am. J. Med.*, 13: 793, 1952.
81. Braunsteiner, H., Falkner, R., Neumayer, A. and Pakesh, F. Makromolekulare Kryoglobulinaemie. *Klin. Wchnschr.*, 32: 722, 1954.
82. Fletcher, A. A., Dauphinee, J. A. and Ogryzlo, M. A. Plasma fibrinogen and the sedimentation rate in rheumatoid arthritis, and their response to the administration of cortisone and adrenocorticotrophic hormone (ACTH). *J. Clin. Investigation*, 31: 561, 1952.
83. MacKay, I. R., Eriksen, N., Motulsky, A. G. and Volwiler, W. Cryo- and macroglobulinemia: electrophoretic, ultracentrifugal and clinical studies. *Clin. Research Proc.*, 3: 104, 1955.

Insulin-Zinc Suspensions*

Further Studies, with Emphasis on Lente Insulin

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RECENTLY, Hallas-Møller and his associates[1–3] reported from the Novo Laboratories in Denmark a new series of long-acting insulins called "lente-preparations." The preparations are based on the principle that insulin in the presence of small quantities of zinc, 1 to 2 mg. per 1,000 units, is insoluble at the pH of the blood provided certain anions such as phosphate and citrate are absent. Presumably, the latter have a greater affinity for zinc than insulin has and thus prevent the interaction between insulin and zinc. Amorphous precipitation of insulin in simple combination with zinc from aqueous solutions occurs at pH 4.5 to 8; crystals form at pH 5 to 6. Aqueous insulin-zinc suspensions are stable at neutral pH and when injected into a subcutaneous depot dissolve slowly, producing a prolonged insulin effect. The degree of prolongation depends primarily upon the physical state of the insulin-zinc suspensions, that is, amorphous or crystalline, and also on the size and shape of the crystals. The amorphous suspensions show time action only slightly more prolonged than that of insulin in solution, whereas the crystalline suspensions are said to behave in true depot-insulin fashion, with duration of effect equal to or greater than that of standard protamine zinc insulin. Supposedly, any desired degree of intermediate effect between these two extremes can be achieved by a proper mixture of the two.

Until now all modifications of insulin with prolonged action in common use in this country have depended upon the combination of insulin with a foreign protein (protamine or globin) to retard absorption from the subcutaneous depots. Phosphate, a suitable buffer at body pH, has been used in various preparations of insulin. The studies of Hallas-Møller et al. previously referred to indicate that it is not necessary to employ a foreign protein to prolong the action of insulin.

Three types of insulin-zinc suspensions have been developed by the Danish investigators for clinical use: semilente, ultralente and lente. Semilente is an amorphous suspension of highly purified insulin with a relatively short range of activity, twelve to sixteen hours. Ultralente is a crystalline suspension of insulin with a very long range of activity extending beyond twenty-four hours. Lente is a mixture containing 30 per cent semilente and 70 per cent ultralente, with a normal action range of about twenty-four hours. The Danish group have found on extensive clinical trial that in the majority of diabetics the twenty-four-hour insulin requirement is satisfactorily met by one daily injection of lente alone. Semilente and ultralente have been employed mainly as mixing components for lente, making it less or more retarded, respectively, in its action. In a few cases semilente and ultralente have been used alone.

For the past four years we have been conducting clinical and chemical studies on the timing of different insulin-zinc suspensions, in collaboration with the Lilly Research Laboratories. One of the chief objectives of this study has been to determine whether these preparations possess any distinct advantage over long-acting insulins now on the market. In a preliminary report[4] we were able to confirm the findings of the Danish group that the action of insulin can be greatly prolonged by simple combination with zinc under proper conditions.

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A crystalline insulin-zinc suspension somewhat similar to the Novo ultralente was found to resemble closely standard protamine zinc insulin in respect to solubility and timing properties.

In the present studies the clinical timing properties of a "lente" preparation somewhat similar to the Novo "lente" insulin described (that is, a mixture containing 70 per cent insulin-zinc suspended in the crystalline form and 30 per cent in the amorphous form) have been compared with those of NPH insulin. The solubility properties of amorphous and crystalline insulin-zinc suspensions and of various mixtures of the two have also been investigated.

CLINICAL COMPARISON OF LENTE INSULIN AND NPH INSULIN

The method has been described in detail elsewhere.[5,6] Briefly, the procedure was as follows: six patients (five women and one man) with severe unstable diabetes were studied on the Metabolic Floor under routine but rigidly controlled conditions throughout the period of investigation. Only patients with the unstable form of diabetes were chosen because it has been shown that differences in insulin timing are observed chiefly in the relatively unstable group of diabetics; differences in timing tend to be minimized in the relatively stable group. The criteria for classification of diabetic patients on the basis of stability have been outlined in a previous paper.[5] Each patient was maintained on a constant weighed diet of identical foods and food values. (Table I.) In each case the diet approximated that which the individual was receiving prior to hospitalization. Lente insulin and NPH insulin were compared in each patient. Insulin was administered hypodermically in a single daily dose before breakfast. The comparisons are based on the blood and urinary sugar response to single daily doses of the given insulin over periods of four or five consecutive days. On changing from one insulin to the other a period of adjustment (two to three days) was allowed before the comparison was made. Changes in insulin requirement necessitated slight adjustments in dose from time to time. Blood sugars were determined[7] four times daily, at 8 A.M., 11:30 A.M., 4:30 P.M. and 9:30 P.M. Twenty-four hour urine samples were collected in four periods: 7:30 to 11:30 A.M., 11:30 to 4:30 P.M., 4:30 to 9:30 P.M. and 9:30 to 7:30 A.M. The fractional specimens were analyzed qualitatively and the twenty-four-hour specimens quantitatively for sugar.[8]

Analysis of the Data. The blood and urinary sugar data of the six unstable diabetics studied are summarized in Table II. The effect of the insulins on the variability of the blood and urinary sugar responses has been used as the basis of comparison. For a full description of this method of analysis, rationale and basic differences from other methods of comparison, see previous publications.[5,6] The total variability among the blood or urinary sugar responses for each patient on each insulin has been divided into two components, intra- and inter-daily variability. The *intra-daily* variation is the variability among the four determinations of blood

TABLE I
SUPPLEMENTARY INFORMATION ABOUT THE DIETS OF THE PATIENTS IN THIS STUDY

Case No. and Patient	Age and Sex	Meals				
		Time	Protein (in gm.)	Fat (in gm.)	Carbohydrate (in gm.)	Calories
1, J. F.	21, F	8 A.M.	15.6	18.6	28.1	
		12 noon	26.6	23.5	47.7	
		3 P.M.	0.5	0.2	10.1	
		5 P.M.	25.8	20.6	51.9	
		9:30 P.M.	7.0	1.8	13.4	
		Totals	75.5	64.7	151.2	1,488
2, H. S.	68, F	8 A.M.	18.9	23.1	46.7	
		12 noon	37.1	33.9	77.0	
		3 P.M.	7.0	0.2	10.2	
		5 P.M.	23.2	25.8	77.3	
		9:30 P.M.	8.2	9.3	18.2	
		Totals	94.4	92.3	229.4	2,126
3, M. A.	44, F	8 A.M.	18.0	32.2	51.3	
		12 noon	33.7	35.1	52.7	
		3 P.M.	0.3	0.1	13.0	
		5 P.M.	28.1	32.3	41.8	
		9:30 P.M.	8.9	10.0	19.2	
		Totals	89.0	109.7	178.0	2,055
4, G. C.	56, F	8 A.M.	15.2	8.0	31.8	
		12 noon	27.1	23.1	44.1	
		3 P.M.	0.5	0.2	10.1	
		5 P.M.	27.0	18.7	50.4	
		9:30 P.M.	4.6	0.2	14.5	
		Totals	74.4	50.2	150.9	1,353
5, S. S.	18, F	8 A.M.	19.3	39.3	60.2	
		12 noon	31.7	26.7	73.7	
		3 P.M.	7.0	0.2	10.2	
		5 P.M.	30.5	29.3	61.8	
		9:30 P.M.	9.1	11.8	29.9	
		Totals	97.6	107.3	235.8	2,299
6, G. M.	56, M	8 A.M.	17.5	30.6	49.9	
		12 noon	31.5	26.7	67.4	
		3 P.M.	7.0	0.2	10.2	
		5 P.M.	33.3	29.2	65.0	
		9:30 P.M.	9.1	11.8	29.9	
		Totals	98.4	98.5	222.4	2,170

TABLE II

SUMMARY OF BLOOD AND URINARY SUGAR DATA

Case No. and Patient	Insulin	Date	Daily Dose (U)	Blood Sugar in mg. % at				Urine Sugar gm./24 hr.
				8 A.M.	11:30 A.M.	4:30 P.M.	7:30 P.M.	
1, J. F.	NPH	4/28/53	50	74	155	38	38	0.93
		29	50	146	184	41	34	2.68
		30	50	78	100	39	34	0.68
		5/ 1	44	127	165	72	54	3.17
		Mean	48.5	106	151	47	40	1.87
	Lente	5/ 4	40	43	102	70	96	0.62
		5	40	177	253	199	201	15.38
		6	40	36	38	44	25	0.55
		7	36	104	129	37	141	3.25
		Mean	39	90	131	88	116	4.95
4, G. C.	NPH	6/15/53	56	140	206	273	182	10.80
		16	52	72	188	150	82	1.50
		17	52	88	191	160	176	3.20
		18	48	138	249	213	156	7.10
		Mean	52	110	209	199	149	5.65
	Lente	6/22	44	314	393	371	307	38.60
		23	44	105	226	284	285	19.00
		24	44	248	348	307	294	26.60
		25	44	203	319	261	254	12.30
		Mean	44	218	322	306	285	24.13
3, M. A.	NPH	6/ 1/53	30	222	256	224	161	32.0
		2	30	247	280	273	198	65.5
		3	30	159	211	218	172	27.8
		4	30	135	172	167	111	57.8
		Mean	30	191	230	221	161	45.78
	Lente	6/ 8	28	127	187	187	100	18.8
		9	26	92	172	198	126	15.9
		10	24	106	169	241	182	19.9
		11	24	251	269	279	223	53.0
		Mean	25.5	144	199	226	158	26.9
5, S. S.	NPH	11/23/53	70	304	264	280	233	92.80
		24	70	243	226	59	103	14.63
		25	70	213	155	180	170	34.47
		26	70	213	121	179	212	78.90
		Mean	70	243	192	175	177	55.20
	Lente	11/30	75	40	170	151	67	12.39
		12/ 1	75	47	147	221	167	21.79
		2	70	261	74	120	49	22.89
		3	65	54	161	172	92	20.12
		Mean	71.3	101	136	166	94	19.30
6, G. M.	NPH	12/ 7/53	56	313	307	71	71	30.72
		8	56	275	367	247	276	64.39
		9	56	270	316	158	237	39.80
		10	56	236	294	63	176	20.00
		Mean	56	274	321	135	190	38.54
	Lente	12/14	60	237	340	327	31	55.90
		15	60	109	262	229	209	34.59
		16	65	279	376	331	288	131.60
		17	70	311	366	275	205	83.50
		Mean	63.8	234	334	291	183	76.40

TABLE II (*Continued*)

Case No. and Patient	Insulin	Date	Daily Dose (U)	Blood Sugar in mg. % at				Urine Sugar gm./24 hr.
				8 A.M.	11:30 A.M.	4:30 P.M.	7:30 P.M.	
2, H. S.	NPH	5/11/53	66	288	300	91	151	37.20
		12	62	384	342	160	211	55.30
		13	62	398	382	292	231	72.30
		14	68	175	248	263	258	34.30
		15	70	137	302	223	259	32.60
		Mean	65.6	276	315	206	222	46.34
	Lente	5/18	70	65	234	204	193	17.90
		19	66	228	280	228	91	35.90
		20	64	280	392	377	346	63.60
		21	60	202	176	226	91	23.50
		22	60	378	365	183	148	52.80
		Mean	64	231	289	244	174	38.74
	NPH	5/25	60	179	165	112	235	34.10
		26	60	347	350	317	252	86.70
		27	60	232	233	126	161	30.30
		28	60	380	337	254	226	65.80
		Mean	60	285	271	201	219	54.23

sugar in a single day. The *inter-daily* variation is the variability among the average blood sugar levels from one day to another. While *intra-daily* variation measures the extent of the variability among the four blood sugar determinations in a single day, it fails to describe the pattern of this variability. It is possible for two insulins to produce no detectable difference in intra-daily variation even though they produce clearly different patterns. In order to describe the pattern of the intra-daily variability or, expressed in other words, the daily pattern of distribution of insulin activity, the deviations from the daily mean blood sugar at the four specified times of the day have been calculated for each patient on each insulin. Precise definitions of the different components of variability and methods of calculation have been published previously.[5] The usefulness of this method in evaluating significant differences in insulin timing has been confirmed recently in England by Paley and his associates.[9,10]

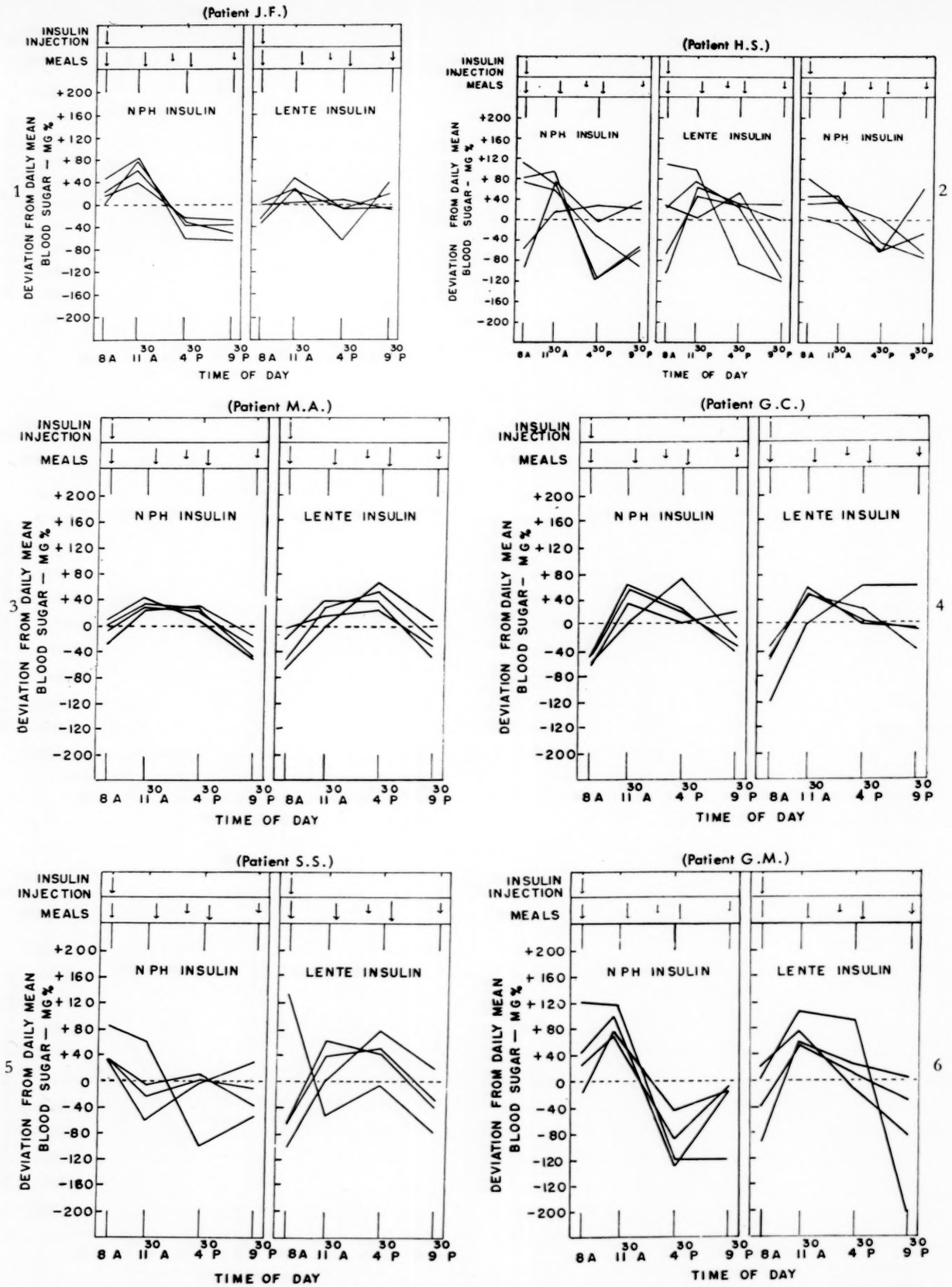
Results. Intra- and inter-daily variation: It was found in previous studies[5] that on the present measurement scale intra-daily variation in blood sugar ranged from about 2.00 in non-diabetics to about 5.00 for the highest value obtained in diabetics. Similarly, the highest value of inter-daily variation obtained in diabetics was 5.50 as compared to a value of 2.50 for non-diabetics.

Table III presents the average values for intra- and inter-daily variation for each patient on each insulin. These values fall within the range expected for unstable diabetics. Individual patients show significantly different levels of intra- or inter-daily variation in blood sugar

TABLE III
AVERAGE INTRA-DAILY AND INTER-DAILY VARIATION IN BLOOD SUGAR (MG. %)

Case No. and Patient	Intra-daily Variation		Inter-daily Variation	
	NPH Insulin	Lente Insulin	NPH Insulin	Lente Insulin
1, J. F.	3.9*	3.2	3.8	4.9
2, H. S.	4.3	4.2	4.8	4.9
3, M. A.	3.5	3.7	4.5	4.6
4, G. C.	3.9	3.9	4.3	4.6
5, S. S.	3.7	4.2	4.6	3.6
6, G. M.	4.3	4.2	4.6	4.7

* Each average is based on four or five consecutive days of observation.



FIGS. 1 to 6. Patterns of distribution of insulin activity (over four consecutive days) of single daily doses of two different types of modified insulin as measured by the patterns of intra-daily variation in blood sugar response at the four specified times of day.

with the two insulins investigated. However, the data do not demonstrate any consistent tendency for either insulin to maintain these measures at levels higher or lower than the other.

Deviations from the daily mean: The results of this part of the analysis are presented graphically in Figures 1 to 7. Figures 1 to 6 illustrate the individual daily patterns of intra-daily variation over periods of at least four consecutive days produced by NPH insulin and lente insulin in each of the six patients. Each figure corresponds to one patient. The average or mean daily pattern for each individual is shown in Figure 7. Each line in Figure 7 represents the average of all days in which the patient received the given insulin.

Inspection of the data reveals at a glance not only that the two insulins produced different patterns in a given patient but also that the same insulin produced different patterns in different patients. Closer inspection reveals that the differences in patterns of blood sugar response produced by the two insulins appear to be fairly consistent and characteristic. In four patients (J. F., H. S., S. S. and G. M., Figs. 1, 2, 5 and 6) NPH displayed some tendency to maintain high levels at 8 A.M., 11:30 A.M., or both, and lower levels at 4:30 P.M. and 9:30 P.M. In these patients lente insulin produced a more nearly level pattern of response, with no conspicuously high or low values (Fig. 1), or reversed the pattern. (Figs. 5 and 6.) Even in patient H. S. (Fig. 2), whose patterns of response were more disorganized, lente insulin appeared to have a slight tendency to produce lower levels at 8 A.M. and higher levels at 4:30 P.M. when compared with NPH. In the remaining two patients, M. A. and G. C. (Figs. 3 and 4), in whom NPH insulin had a tendency to produce slightly lower levels at 8:00 A.M. and 9:30 P.M. and higher levels at 11:30 and 4:30 P.M., lente insulin either produced few perceptible changes (Fig. 4) or slightly exaggerated the NPH pattern. (Fig. 3.) When the mean patterns of response to the two insulins are compared in the group as a whole (Fig. 7), it will be noted that the over-all patterns of response of NPH insulin and lente insulin are quite similar. However, the same differences, although somewhat more damped, are apparent. Lente insulin has a tendency to maintain slightly lower levels at 8 A.M. and 9:30 P.M., and slightly higher levels at 11:30 A.M. and 4:30 P.M. than does NPH.

Comment. When the two insulins are compared as to their ability to control intra- and inter-daily variability, no large differences appear. This indicates that large differences in predictability of action and consistency of response between the two insulins have not been detected. Lente insulin appears to suffer from the same limitation as NPH insulin. Although the present group of patients studied is very small, it is unlikely that a study of a larger group would reveal differences of clinical significance in view of previous studies of a much larger scope involving a comparison of several insulins.[5] It was found that the several insulins studied showed no differential ability to control the extent of the variability of blood and urinary sugar. This has been confirmed recently by Paley and his associates in England.[9,10] The present findings serve only to lend additional support to the hypothesis that the type of insulin may influence the pattern of intra-daily variability but cannot control the extent of variability of the blood and urinary sugar responses or, in other words, *stability*. In spite of the best possible control of external factors (diet, environment and insulin) a certain basic instability may remain which, to a certain extent, appears to be dependent upon endogenous factors in the patient.

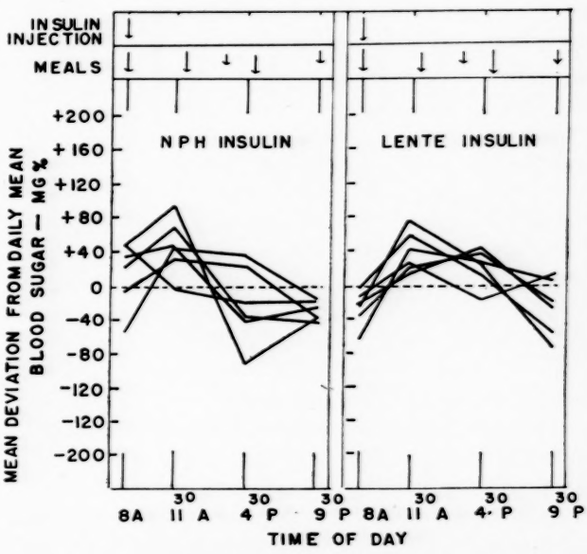


FIG. 7. Mean daily patterns of distribution of insulin activity of single daily doses of two different types of modified insulin as measured by the mean pattern of intra-daily variation in blood sugar response in each of the six patients with unstable diabetes. Each line represents the average over-all days in which each patient received the given insulin.

The patterns of intra-daily variation of the blood sugar produced by lente and NPH insulins suggest that the pattern of distribution of insulin activity of lente is similar to but a trifle slower than that of NPH. However, even though the differences are relatively minor when considered

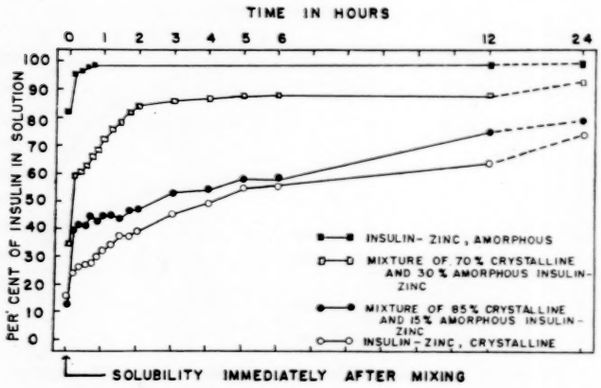


FIG. 8. Solubility curves of different types of insulin-zinc suspensions in 60 per cent pooled human sera at 37°C. and pH 7.3.

in the group as a whole, the findings indicate that the two insulins are not always directly interchangeable in unstable diabetics. In those patients in whom NPH has a tendency to produce relatively higher levels of blood sugar in the night and in the morning, lente may produce a more nearly level pattern of response. In those patients in whom NPH has a tendency to produce higher levels during the day and lower levels at night and in the morning, lente may exaggerate the pattern.

COMPARISON OF SOLUBILITY PROPERTIES OF VARIOUS TYPES OF INSULIN-ZINC SUSPENSIONS IN SERUM

The solubility properties of four different types of insulin-zinc suspensions were compared in 60 per cent pooled human sera at pH 7.4 and 37°C., according to a method described previously.[11] The preparations studied include: (1) a suspension of insulin-zinc in the amorphous state, somewhat similar to the Novo "semilente" insulin, and bearing the Lot No. 3113; (2) a suspension of insulin-zinc in the crystalline state, somewhat similar to Novo "ultralente" insulin, and bearing the Lot No. 3095; (3) a mixture containing 70 per cent insulin-zinc in the crystalline and 30 per cent in the amorphous state, somewhat similar to the Novo "lente" insulin, bearing the Lot No. 3089; (4) a mixture containing 85 per cent insulin-zinc in the crystalline state and 15 per cent in the amorphous state and bearing the Lot No. 3097. Hereinafter, these preparations will be referred to as semilente, ultralente, lente and type 85/15, respectively.

The solubility curves of these insulins are shown in Figure 8. The values plotted are averages of duplicate determinations at three different times. Definite and characteristic differences in the solubility curves of each individual type are noticeable. A graded series of responses was obtained with amorphous insulin-zinc at one end and insulin-zinc crystals at the other end of the solubility scale. Most of the amorphous insulin-zinc dissolved almost instantaneously in the *in vitro* serum system, the remainder going into solution in a matter of minutes. In contrast, the insulin-zinc crystals went into solution very slowly and by the twenty-fourth hour approximately 25 per cent was still undissolved. The two mixtures dissolved at rates intermediate between these two extremes, the rates being proportionate to the ratio of amorphous to crystalline insulin-zinc.

Comment. It has been pointed out previously[4,11] that the solubility rates indicated by this type of study are not to be construed as identical with absorption times of the different insulins from subcutaneous depots. They are useful only for comparative purposes. Nevertheless, the correlation between the solubility curve and relative clinical timing of a given preparation suggests that the timing of such a preparation is a direct function of its solubility properties. On the basis of cumulative experience, it is possible to obtain a rough estimate of the relative timing of a given preparation from its solubility curve alone. The fairly close correlation between the relative clinical timing and relative solubility characteristics of lente and ultralente insulin are in agreement with such a view. Thus the results suggest that it is possible to obtain a spectrum of insulin-zinc suspensions with graded clinical timing properties, the amorphous suspension falling at one end and the crystalline suspension at the other end of the timing scale. Theoretically, practically any desired intermediate effect between these two extremes may be obtained by proper mixture of the two.

GENERAL DISCUSSION

Before discussing the insulin-zinc suspensions in relation to long-acting insulins now on the

market it may be pertinent to digress a moment to discuss briefly the three major types of blood sugar responses to successive, single, daily doses of a modified insulin. It may be well also to discuss briefly some important inter-relationships among type of response, type of patient and type of insulin.

The first type of blood sugar responses or level pattern of response is characterized by the absence of conspicuously high or low levels of blood sugar throughout a twenty-four-hour period and represents a balanced distribution of insulin. The second, or convex pattern, with higher levels of sugar during the day and lower levels at night and in the morning, represents an unbalanced distribution of insulin activity characterized by too little activity during the day and too much at night. The third, or concave pattern, with lower levels during the day and higher levels at night and in the morning, is in certain respects opposite to the second type, that is, too much activity during the day and too little at night. The convex pattern results in a tendency toward postprandial hyperglycemia and glycosuria on the one hand and hypoglycemia overnight on the other. In contrast, the concave pattern tends to result in hypoglycemia during the middle of the day and high levels overnight and in the morning.

It is estimated that at least half of the total diabetic population requires exogenous insulin in addition to dietary restriction for adequate regulation.[12] About 50 per cent of those taking insulin, or approximately 75 per cent of the total, are estimated to belong to the relatively *stable* group.[5] The remaining 25 per cent or less comprise the relatively *unstable* group. The stable form is seen mainly in older individuals, especially middle-aged obese women, while the unstable form is seen especially in younger individuals but may occur at any age level. The first, or level, pattern of response is typical of the stable group regardless of type or amount of insulin used. In contrast, all three types of response may be elicited by the same insulin in different individuals in the unstable group. This is well illustrated by the varying responses to the insulins used in the present study. In our experience NPH insulin may be expected to produce the level or nearly level patterns of response more often than the convex or concave types of response in the unstable group. Standard protamine zinc insulin usually produces a convex pattern of response, while globin insulin with zinc has a strong tendency to produce a concave pattern.

Heretofore, interest in new insulins has centered chiefly on the development of a preparation able to meet the needs of most patients. It is apparent from the foregoing as well as from previous studies that no single preparation with fixed timing is capable of producing a level or nearly level pattern of response or, in other words, optimal timing in all patients. A well timed preparation, for example, NPH insulin or lente insulin, may be capable of achieving satisfactory if not optimal timing in the majority of patients. However, a certain number will remain for whom an insulin or insulins with different timing characteristics would be more suitable. For these reasons it might be more desirable to develop a selected series of insulins to cover the spectrum of timing requirements of the diabetic population rather than to concentrate on one preparation.

At present there are five standard insulins on the American market, exclusive of lente insulin. The first two (regular insulin and crystalline insulin) are unmodified and have a relatively short duration of effect. The other three (protamine zinc insulin, globin insulin with zinc and NPH insulin) have been modified with protamine or globin to achieve a more or less prolonged effect. It is true that, used alone in single or multiple daily doses, or in various combinations, they are capable of achieving satisfactory regulations in most if not all patients. However, this is still a makeshift program involving different insulins in different doses and in some cases multiple daily injections. Furthermore, these insulins form a heterogeneous group with reduplication, overlap or wide separation in timing properties. The timing of these modified preparations is more or less fortuitous, being fixed by the physical characteristics of the preparations. Therefore, the potential advantages of the insulin-zinc suspensions would seem to lie in the possibility of developing a homogeneous, uniform, small series of preparations carefully selected to span the spectrum of timing needs of the diabetic population. This would afford the opportunity of testing the hypothesis that most patients can be treated by one single daily injection of a suitably timed insulin preparation. Such a series could replace the existing modified insulins advantageously and thus simplify and improve rather than confuse the commercial insulin situation further. It is beyond the scope

of this paper to discuss the pro's and con's of such an approach. In the final analysis the comfort and convenience of the patient should be the deciding factors.

It may be worth while to re-emphasize at this point that while a suitably timed insulin may add to the comfort and convenience of the patient, it does not appear to affect stability. Hence more attention to the basic metabolic factors involved in stability, rather than mere attempts to improve existing fixed-timing types of insulins, would seem appropriate.

SUMMARY

Further studies on a new series of long-acting insulin preparations, referred to as "insulin-zinc suspensions," are reported. These preparations, developed in the Novo Laboratories in Denmark by Hallas-Møller et al., are based on the principle that insulin in the presence of small quantities of zinc is insoluble at the pH of the blood. Insulin in simple combination with zinc can be precipitated from aqueous solutions in either the amorphous or crystalline form. Amorphous insulin-zinc suspensions have a time action only slightly more prolonged than that of unmodified insulin, whereas insulin-zinc crystals have a greatly prolonged effect.

A mixture containing 70 per cent insulin-zinc crystals and 30 per cent amorphous insulin-zinc, designated "lente" insulin, was compared with NPH insulin in each of six patients with unstable diabetes. The blood and urinary sugar responses to successive single daily doses of lente insulin and NPH insulin over periods of several consecutive days were determined in each patient. The insulins were compared in each patient as to their ability to control the extent, or to influence the pattern, of variability of blood sugar response.

There was no consistent tendency for one insulin to maintain intra- or inter-daily variability in blood sugar at levels higher or lower than the other. The patterns of intra-daily variability produced by the two insulins in the group as a whole suggest that the daily distribution of insulin activity of lente insulin is similar to but slightly slower than that of NPH insulin. However, the two insulins may not always be directly interchangeable in the individual patient. In those patients in whom NPH has a tendency to produce a concave pattern of response with lower sugar levels during the day and higher levels in the night and morning, lente may produce a more nearly level pattern. In those patients in whom NPH may produce a convex pattern of response with higher sugar levels during the day and lower levels at night, lente may exaggerate the pattern.

The solubility properties of amorphous insulin-zinc, insulin-zinc crystals and mixtures of the two were studied in a standardized, serum-containing, *in vitro* system. A graded series of solubility curves was obtained. Amorphous insulin-zinc dissolved rapidly whereas insulin-zinc crystals dissolved at a slow rate. The solubility curves of mixtures of the two were intermediate between these two extremes. The high correlation between the relative solubility properties and the relative clinical timing of an insulin preparation observed in these and in previous studies suggest that it is possible to obtain a variety of insulin-zinc suspensions with different durations of activity suitable for clinical use.

The three major patterns of sugar response to successive single daily doses of any given insulin and some of the inter-relationships among type of response, type of patient and type of insulin are discussed. The potentialities of insulin-zinc suspensions as compared with long-acting insulins now on the market are considered in terms of these inter-relationships.

REFERENCES

1. Hallas-Møller, K., Petersen, K. and Schlichtkrull, J. Crystalline and amorphous insulin-zinc compounds with prolonged action. *Ugesk. f. læger*, 113: 1761–1767, 1951.
2. Hallas-Møller, K., Petersen, K. and Schlichtkrull, J. Clinical investigations on new insulin preparations with a prolonged action—insulin-zinc preparations—used in one daily injection. *Ugesk. f. læger*, 113: 1767–1771, 1951.
3. Hallas-Møller, K., Petersen, K. and Schlichtkrull, J. Crystalline and amorphous insulin-zinc compounds with prolonged action. *Science*, 116: 394–398, 1952.
4. Izzo, J. L., Gabiga, A. M. and Hoffmaster, J. Pharmacologic and clinical studies on two new types of long-acting insulins with special reference to zinc insulin preparations (Novo): a preliminary report. *Diabetes*, 2: 358–364, 1953.
5. Izzo, J. L., Crump, S. L. and Kunz, W. A clinical comparison of modified insulins. *J. Clin. Investigation*, 29: 1514–1527, 1950.
6. Izzo, J. L. and Crump, S. L. Symposium on the design and interpretation of clinical experiments with drugs, New York City, April 14, 1952. A clinical comparison of modified insulins. I. The clinical problem. II. The biometrical aspects of the problem. *Biometrics*, 6: 206–217, 1952.

7. Somogyi, M. Determination of blood sugar. *J. Biol. Chem.*, 160: 69, 1945.
8. Shaffer, P. A. and Hartman, A. F. Iodometric determination of copper and its use in sugar analysis. II. Methods for the determination of reducing sugars in blood, urine, milk and other solutions. *J. Biol. Chem.*, 45: 365, 1921.
9. Fryers, G. R., Paley, R. G. and Tunbridge, R. E. The design of insulin trials. A polylysine insulin. *Lancet*, 2: 782–784, 1954.
10. Paley, R. G. The design of insulin trials. Insulin-zinc suspension (Lente). *Lancet*, 2: 784–787, 1954.
11. Izzo, J. L. and Kunz, W. Studies on modified insulins. I. Comparison of time-action curves in stabilized patients with constant hyperglycemia and glycosuria. II. Comparison of solubility properties in water and sera. *Proc. Am. Diabetes A.*, 9: 225–243, 1949.
12. Colwell, A. R. Treatment of diabetes. Selection of technic according to severity. *Diabetes*, 2: 262–267, 1953.

Reviews

Cryo- and Macroglobulinemia*

Electrophoretic, Ultracentrifugal and Clinical Studies

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THE production of plasma globulins with unusual properties, such as cryoglobulins[1] and macroglobulins,[2,3] may occur in various diseases involving the reticulo-endothelial system. Cryoglobulins are characterized by reversible cold precipitation, while macroglobulins are proteins of very high molecular weight detectable only by ultracentrifugal analysis. Occasionally, an abnormal plasma protein may be both a cryoglobulin and a macroglobulin.

In this paper five patients with macro- or cryoglobulinemia, or combinations thereof, are reported. The physicochemical properties of cryo- and macroglobulins and their clinical significance are discussed. The lack of uniform characteristics as well as the frequent absence of symptoms directly attributable to these abnormal globulins is emphasized.

CASE REPORTS

CASE 1. *Cryo- and macroglobulinemia with pancytopenia:* S. B., a sixty-nine year old white woman, was admitted to King County Hospital on several occasions between 1950 and 1954 for febrile respiratory infections, often associated with pneumonic infiltrates. She had been well until 1949 when an illness developed, diagnosed elsewhere as "glandular fever," with enlarged tender cervical nodes, fever, chills and malaise. During a subsequent hospitalization for pneumonia hepatosplenomegaly, lymphadenopathy, anemia and neutropenia were discovered. The patient had had an attack of anginal-like pain in 1950, and erythema nodosum developed in 1951. Hyperglobulinemia was first noted in 1952 and cryoglobulinemia in 1954. The patient disclaimed Raynaud's phenomenon and cold intolerance. On physical examination at each admission she appeared thin, pale and chronically ill. A few petechial spots and recent bruises were frequently observed. Submandibular, cervical and axillary lymph nodes were slightly enlarged. Retinal examination in 1954 revealed multiple hemorrhages. Severe epistaxis required hospitalization in June, 1954.

Laboratory examinations carried out between 1952 and 1954 showed the following: the hematocrit varied from 37 to 40. Leukocyte count ranged between 2,000 and 4,000, with neutropenia varying between 3 and 13 per cent. During bacterial infections, a mild polymorphonuclear leukocytic response was observed. The platelet count was 90,000 to 150,000, and reticulocytes 1 to 2 per cent. The blood sedimentation rate could not be determined due to gel formation of the plasma. The Coombs test gave a negative result as did a lupus preparation from the peripheral blood. Bone marrow biopsy showed a mild increase in myeloid elements and a slight increase in mature plasma cells (5 per cent); there was no increase of small naked lymphocytes.§ Survival time of normal transfused erythrocytes was eighty days (Ashby method). Urinary protein was 0–80 mg. per day; Bence-Jones protein was not found. The total serum protein in February, 1954, was 9.3 gm. per cent, albumin 2.1 gm. per cent, globulin 7.2 gm. per cent (method of Wolfson et al.[5]). Massive cryoglobulinemia and macroglobulinemia were present. Bromsulfalein retention at forty-

§ According to Waldenström[4] large numbers of small atypical lymphocytes almost devoid of cytoplasm are usually found in macroglobulinemia.

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‡ John and Mary R. Markle Scholar in the Medical Sciences.

five minutes was 5.6 per cent. The cephalin flocculation was 4+ in forty-eight hours. Tuberculin tests and serologic tests for syphilis gave negative results. Radiologic examination of the chest and bones (including hands and skull) were normal.

Cortisone therapy was instituted in February, 1954, with no clinical or hematologic improvement. Because of neutro- and thrombocytopenia splenectomy was performed in March, 1954. Microscopic examination of the spleen showed a cellular pulp, active reticular tissue and multiple hemosiderin deposits in macrophages. Surgical liver biopsy was normal. The blood granulocytes and platelets rose slightly following splenectomy, but febrile episodes recurred. There were severe headaches and several syncopal attacks. In November, 1954, the patient suddenly became comatose and died within a few days. Prior to her death cryoglobulins and macroglobulins were found in high concentration in the cerebrospinal fluid. The significant gross autopsy finding was an intracerebral hemorrhage originating in the left thalamic region.

Comment. An elderly woman exhibited pancytopenia, splenomegaly and massive cryo- and macroglobulinemia. The clinical course was characterized by recurrent infections. Sudden death occurred due to intracerebral hemorrhage.

Case II. *Macroglobulinemia:* This sixty-seven year old white man (R. M.) has been admitted twenty times since March, 1937, to the U. S. Public Health Hospital in New Orleans.* He had been treated with mapharsen for latent syphilis in 1937. In 1940 leg edema, exertional and nocturnal dyspnea, and epistaxis were first noted. His blood pressure was 184/122 at that time. These complaints continued over the years. In 1947 his hemoglobin was 13 gm. per cent, hematocrit 47 and white count 5,600, with 63 per cent neutrophils and 34 per cent lymphocytes. The patient was hospitalized in 1949 for dizziness, headache, epistaxis, slurred speech and a cloudy mental state. The blood pressure then was 190/98. A dilated fixed pupil and elevated spinal fluid pressure were found. In 1950 a perforated nasal septum and a prepyloric gastric ulcer were discovered. Several admissions for ulcer treatment followed in 1950 and 1951. Cephalin flocculation was 2+ in December, 1950. In February, 1952, a three-day episode of hemoptysis occurred and a diagnosis of bronchiectasis was considered. The white count was 7,700 and hemoglobin 12 gm. per cent. In early 1954 the patient was readmitted for slow continuous nosebleeds and drowsiness. His blood pressure fluctuated from 180/90 to 115/60. The sedimentation rate was 18 to 22. A mild normochromic, normocytic anemia (hemoglobin 8.5 to 11 gm. per cent) and leukopenia (leukocyte count 4,500 to 6,000) were discovered. Reticulocytes were 1.8 per cent. The serum non-protein nitrogen was 38.4 mg. per cent. Serum protein concentration was 7.2 gm. per cent with albumin 3.5 gm. and globulins 3.7 gm. (method of Wolfson et al.[5]). Gastrojejunostomy for antral obstruction from the ulcer was performed in February, 1954. No enlargement of liver or spleen was found at operation.

The patient was readmitted in September, 1954, for weakness. He was found to be very lethargic. Dyspnea was successfully treated with aminophylline. There was no ankle edema. A few small axillary lymph nodes were observed. The spleen was not enlarged. X-rays of the skull were normal. An osteochondroma of the humerus was an incidental finding. There was significant pancytopenia (leukocyte count 3,350, 37 per cent polymorphonuclears, 52 per cent lymphocytes, 12 per cent monocytes; erythrocyte count 2.51 million; hemoglobin 7 gm per cent; hematocrit 24; platelets 170,000; reticulocytes 1.6 per cent). The corrected sedimentation rate was 23. Prothrombin time was 100 per cent. Coombs test gave negative results and red cell osmotic fragility was normal. Bone marrow examination revealed normoblastic hyperplasia and an increase of lymphocytoid cells; many of the latter were quite naked without cytoplasm, others had spindle-shaped cytoplasm. A few atypical plasma cells with nucleoli were observed. Urinalysis revealed a specific gravity of 1.022, protein 75 mg. per cent (1+), a few granular and hyaline casts, 15 to 20 white cells and 2 to 4 red cells per high power field. Repeated examinations for Bence-Jones protein gave negative results. The serologic test for syphilis (VDRL) also gave negative results. By the method of Wolfson et al.,[5] total serum protein was 12.3 gm. per cent with 3.0 gm. albumin, 0.29 gm. α_1 globulins, 0.97 gm. α_2 globulins, 0.45 gm. β globulins and 7.6 gm. γ globulins. Cephalin flocculation test was 3+ in forty-eight hours and thymol turbidity 12.8 Maclagan units. Ultracentrifugal analysis showed marked macroglobulinemia. The Sia water test[7] gave negative results.

Comment. A sixty-seven year old man with a syphilitic history had hypertension with symptoms of mild edema and dyspnea for several years. Epistaxis, lethargy, pancytopenia and marked hyperglobulinemia developed. Macroglobulinemia but no cryoglobulinemia was then discovered.

Case III. *Cryoglobulinemia in myeloma:* S. A., a white male laborer aged seventy-six, born in Iceland, was first admitted to King County Hospital, Seattle, in July, 1949, for a two-stage suprapubic prostatectomy. Anemia (hemoglobin 9.3 gm. per cent) and elevated blood sedimentation rate (120 mm. per hour, corrected, Westergren) were unexplained. The second admission (November, 1952) was for pain in the low back and right side of chest, cough, excessive sputum and fever. The diagnosis at that time was right lower lobar pneumonia and arteriosclerotic heart disease. Laboratory data included: leukocyte count 26,000 (later falling to 9,500), hemoglobin 12.3 gm. per cent,

* This case is reported through the courtesy of Drs. Linden E. Johnson and Joseph H. Davis, U. S. Public Health Service Hospital, New Orleans, La.

serum albumin 1.6 gm. per cent and serum total globulins 5.3 gm. per cent (micro-Kjeldahl method[6]).

Within a week after the patient was discharged he was readmitted with pain and tenderness of the ribs, and backache. Bone marrow aspiration showed numerous plasma cells, typical of multiple myeloma. Bence-Jones urinary protein was not found. Cryoglobulinemia was present. No history was obtained of abnormal bleeding tendency or Raynaud's phenomenon. Radiography showed an 8 cm. calcified shell in the liver, presumably an hydatid cyst. Apart from a single radiolucency in the second left lumbar transverse process, bone survey showed no other myeloma lesions. Urethane treatment was begun.

The fourth admission in January, 1953, was for severe pain in the bones. The patient survived pneumococcal meningitis and septicemia but thereafter deteriorated gradually; death occurred in March, 1953. Autopsy was not performed.

Comment. This elderly man had multiple myeloma and asymptomatic cryoglobulinemia. Multiple infections finally resulted in death.

CASE IV. *Cryoglobulinemia in cirrhosis and myeloma:* M. H., a seventy-one year old white woman, was admitted to King County Hospital with pain in the chest and fever. There was a one-year history of anorexia and a forty-pound weight loss. A diagnosis of mild bronchopneumonia was made; this illness responded well to penicillin. Physical findings included a marked firm splenomegaly, slight hepatomegaly, fetor hepaticus, liver palms, spider angiomas, and prominent abdominal veins with a caput medusae. There was no history of alcoholism. Laboratory data revealed: hematocrit 30.0, erythrocytes 2.7 million, platelets 66,000, leukocytes 1,300 to 2,150. The survival time (Ashby method) of normal transfused cells was fifty days. The tourniquet test gave positive results and the bleeding time was fourteen minutes. The blood sedimentation rate (corrected) was 54 mm. per hour. The bromsulfalein retention was 21.5 per cent. The serum albumin concentration was 2.6 gm. per cent, and serum globulin 4.2 gm. per cent (method of Wolfson et al.[5]). The cephalin flocculation was 3+ in forty-eight hours. Esophagoscopy revealed varices.

In February, 1952, splenectomy and splenorenal shunt were performed. The spleen weighed over 1,000 gm. and microscopically showed the typical changes of prolonged congestion. Surgical liver biopsy confirmed a coarsely nodular portal cirrhosis.

The patient improved postoperatively. However, the spider angiomas, liver palms, caput medusae and fetor hepaticus persisted. Laboratory tests in February, 1953, showed: hematocrit 43, platelets 172,000 and leukocytes 6,500. The life span of transfused erythrocytes was normal at this time. The serum albumin was 2.8 gm. per cent, serum globulin 6.0 gm. per cent, cephalin flocculation 1+ in forty-eight hours and bromsulfalein retention 24 per cent. A sternal bone marrow aspirate revealed marked plasma cell infiltration (85 per cent) with immature plasma cells, characteristic of multiple myeloma. At this time cryoglobulins were first noted in the serum. The patient had never noticed Raynaud's phenomenon or abnormal bleeding tendencies.

Comment. An elderly woman had non-alcoholic portal cirrhosis, portal hypertension and pancytopenia. A splenorenal shunt was performed with good results. Myeloma cells and cryoglobulins were first discovered following operation.

CASE V. *Idiopathic cryoglobulinemia:* In 1954, J. Y.,* a fifty year old man, became chilled during a fishing trip and multiple petechial hemorrhages of the legs and swelling of the knees developed. Subsequently, repeated attacks of purpura involving buttocks and legs occurred when the patient was exposed to cold. Symptoms for the past year included marked dryness of the mouth and nose (Sjögren's syndrome), increasing dyspnea, ankle swelling and arthralgias of shoulders and knees without joint deformity.

Physical examination revealed numerous purpuric lesions over the dorsum of the hands and lower extremities, and brownish pigmentation. The blood pressure was 160/100. Liver, spleen and lymph nodes were not palpable. Laboratory investigations revealed a normocytic anemia, hematocrit of 36, leukocyte count of 12,800 with normal differential, and a normal platelet count. The clotting profile was normal except for repeatedly positive reactions to tourniquet tests. Cryoglobulinemia without macroglobulinemia was found. Sternal bone marrow aspiration showed 10 per cent of atypical plasma cells. The blood urea nitrogen was 36 mg. per cent. Serum total protein was 6.8 gm. per cent, albumin 3.3 gm. per cent, globulin 3.5 gm. per cent (micro-Kjeldahl method[6]). Urinalysis revealed 4+ proteinuria with many red cells, hyaline and granular casts; Bence-Jones protein was absent. A skeletal survey for bone lesions was negative.

Comment. A middle aged man with mild azotemia and plasmacytosis of the bone marrow has had symptoms from cryoglobulins (cold sensitivity with purpura) for nine years.

METHODS

Blood was collected while patients were in the fasting state, and serum was separated from the cells within one hour. Protein estimations were carried out by micro-Kjeldahl nitrogen determination[6] except where specified. Semi-quantitative determinations of cryoglobulin were made after allowing serum to remain in a hematocrit tube at 4 to 5°C. for three days; following centrifuging for thirty minutes, 3,000 r.p.m. at 4°C., the per cent volume of the sedimented cryoglobulin was read as a "cryocrit." (Fig. 1.) In each case one to two drops of serum were added to a test tube of distilled water (modified Sia[7] or Brah-

* Referred to us through the courtesy of Dr. Quin de Marsh.

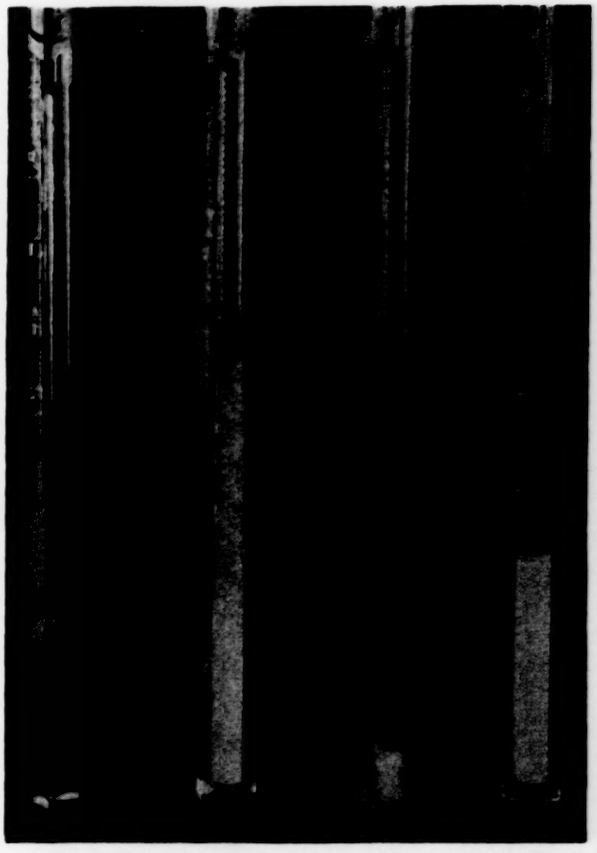
machari[8] test); according to Waldenström,[4,9] a cloudy precipitate suggests the presence of macroglobulins. Technics of free* and paper† electrophoresis have been described previously.[10] Ultracentrifugal analyses were performed with a Spinco model E ultracentrifuge at 22–27°C. after dilution of the sample in 0.2 M saline.‡ The dial setting for speed was 59,780 r.p.m. Exposures were taken seven minutes after reaching top speed and thereafter at intervals of sixteen minutes. The sedimentation coefficients represent directly determined values. The concentrations of the various ultracentrifugal components were estimated from planimetric measurements of the patterns and corrected for the dilution effect arising from the sector shape of the cell.

CRYOGLOBULIN AND MACROGLOBULIN STUDIES

Cryo- and Macroglobulinemia with Panoytopenia. Case 1: The cryoglobulin in this case formed a light amorphous flocculus in both serum and plasma, this change commencing as cooling reached 26°C. The amount of cryoglobulin increased progressively over the course of the illness; the cryocrit increased from 28 to a final reading just prior to death of 80, at which time the serum almost completely coagulated upon cooling. The final approximate serum cryoglobulin concentration was 7.5 gm. per cent.

Electrophoretic analyses: These were performed on whole serum,§ a solution of isolated washed cryoglobulin, "cryoglobulin-free" serum and cerebrospinal fluid. The patterns for whole serum showed two components in the fibrinogen-gamma globulin region by paper electrophoresis, and in the ascending limb pattern by free electrophoresis. (Fig. 2A and D, Table 1.) The mobility of the major spiked component approximated that of fibrinogen, being intermediate between that of normal beta and gamma globulins. Electrophoresis of cerebrospinal fluid yielded similar findings. By both paper and free electrophoretic technics, analyses of cryoglobulin-free serum revealed a faint to absent Cr_1 component and only a small Cr_2 component. (Fig. 2B and E, Table 1.) These findings suggested that both components of the cryoglobulin complex participated in the cold precipitation. The isolated cryoglobulin was analyzed after washing twice with distilled water. With paper electrophoresis it remained at the point of origin (between the beta and gamma globulin areas) and two components could not be identified. (Fig. 2F.) When dissolved in and dialyzed against phosphate buffer pH 3.1, two peaks similar to those of the cryoglobulin in whole serum could be identified in both limbs by free electrophoresis. (Fig. 2C.) Analysis in a different medium (M/10 sodium diethylbarbiturate) likewise showed two components. (Table 1.)

Ultracentrifugal analyses: Both whole serum and cerebrospinal fluid patterns showed, besides the



A B C D

FIG. 1. Cryocrits of various serums demonstrating variable degrees of cryoglobulinemia. A, case of disseminated lupus erythematosus showing minimal cryoglobulinemia typical of numerous chronic inflammatory diseases. (Cryocrit = 1). B and D, Case 1, showing variable amounts of the cryoglobulin observed at different times in this illness. (D = cryocrit of 31 in February, 1954; B = cryocrit of 60 in September, 1954.) C, Case v (Cryocrit = 8).

* In the usual barbiturate buffer (pH 8.6, $\mu = 0.1$) cryoglobulins are usually soluble at 0°C.

† Analyses involving cryoglobulins were performed at room temperature.

‡ In Case 1, additional analyses on the poorly soluble cryoglobulin were performed in 0.2 M saline—0.01 M sodium diethylbarbiturate, and in barbital buffer pH 8.6 ionic strength 0.1.

§ Following dilution in and dialysis against barbiturate buffer at 4°C. A very small amount of precipitated cryoglobulin was removed by centrifuging; this precipitate was analyzed ultracentrifugally.

TABLE I

PROPERTIES OF CRYO- AND MACROGLOBULINS: ELECTROPHORETIC AND ULTRACENTRIFUGAL DATA

Case No.	Material Analyzed	Free Electrophoresis			Paper Electrophoresis	Percentage Composition by Ultracentrifugal Analysis		
		Buffer $\mu = 0.1$	Description of Pattern	Mobility Cryoglobulin $cm^2\ volt^{-1}\ sec^{-1} \times 10^5$	Barbital Buffer pH 8.6 $\mu = 0.1$	A (approximately 4.5S)	G (approximately 7S)	M (15S or greater)
1, S. B.: Cryo- and macroglobulinemia with pancytopenia	Whole serum	Barbital pH 8.6	Asc. two cryoglobulin components in ϕ, γ positions	Cr_1 -2.1 Cr_2 -1.4	Two components in γ globulin area = Cr_1 and $Cr_2 + \gamma$	41	21	6(17S) 17(20S) 15(≧28S)
			Desc. single cryoglobulin spike in γ globulin area	$Cr + \gamma$ -1.7	.....	..	.....	.....
	Supernate	Barbital pH 8.6	Cr_1 absent Small flat γ	γ -1.4	Cr_1 band almost absent	74	21	5(17S)
	Isolated cryoglobulin	Phosphate pH 3.8	Two peaks, spike and hump, both limbs	Cr_1 $+4.8$ Cr_2 $+3.2$	Single band, remaining at point of application, between β and γ areas	..	.....	.....
		M/10 sodium diethylbarbiturate pH 9.7	Two components present, both limbs	Cr_1 -3.0 Cr_2 -2.0	.....	..	30	58(18S) 12(≧27S)
	Distilled water precipitate	Barbital pH 8.6	Asc. two components present	Cr_1 -1.6 Cr_2 -0.8	Single band, remaining at point of application, between β and γ areas	..	23	41(20S) 36(≧27S)
			Desc. single component	$Cr + \gamma$ -1.4	.....	..	.....	.....
	C.S.F.	Barbital pH 8.6	Asc. two cryoglobulin components	Cr_1 -2.0 Cr_2 -1.4	.....	48	18	6(16S) 15(19S) 13(≧27S)
			Desc. single cryoglobulin spike in γ globulin area	$Cr + \gamma$ -1.8	.....	..	.....	.....

2, R. M.: macroglobulinemia	Whole serum	Barbital pH 6.6	Single symmetric spiked peak in γ globulin area resembling myeloma	γ = macro globulin -1.5	Narrow dense band in γ globulin area, as seen in multiple myeloma	35	8	53(15S) 4(20S)
3, S. A.: cryoglobulinemia in myeloma	Whole plasma (oxalated)	Barbital pH 8.6	Technically not possible	.....	Typical myeloma pattern: dense γ band = 56% total protein	..	.....	.....
		0.2M glycine in 0.1N NaOH, pH 9.67	Tall spiked single γ peak	γ=Cr -2.1	.....	..	.....	.....
	Supernate	Barbital pH 8.6	Asc. tall spiked single γ peak	γ=Cr -1.1	Dense γ band, typical of myeloma	47	49	4(20S)
			Desc. γ as broad low hump	γ=Cr -0.7 to -1.2	.....	..	.....	.....
	Lower syrupy layer	Barbital pH 8.6	.....	.....	Cryoglobulin as dense band in γ area	6	92 2(9S)	.....
	Isolated cryoglobulin	Acetate pH 4.65	Single sharp symmetric peak, both limbs	Cr $+4.0$	.....	..	.....	.....
	Wolfson, Cohn γ precipitate[5]	M/20 sodium diethylbarbiturate pH 9.76	Homogeneous single peak	-2.85	.....	..	.....	.....
4, M. H.: cryoglobulinemia in cirrhosis and myeloma	Whole serum	Barbital pH 8.6	Tall spiked γ_1 peak = cryoglobulin	Cr -1.8	Dense discrete band in γ area = 43% of total serum protein	53	43	4(17S)
	Isolated cryoglobulin	Barbital pH 8.6	Single sharp symmetric peak	Cr -1.8	.....	..	100	.....
5, J. Y.: idiopathic cryoglobulinemia	Whole serum	Barbital pH 8.6	Small rounded cryoglobulin peak intermediate between β and γ	Cr -2.5	Discrete cryoglobulin band between β and γ areas; band still detectable in "cryoglobulin free" supernate	80	17	3(17S)
	Isolated cryoglobulin	Barbital pH 8.6	Small, single, hump-shaped peak	Cr -2.5	.....	..	100	.....

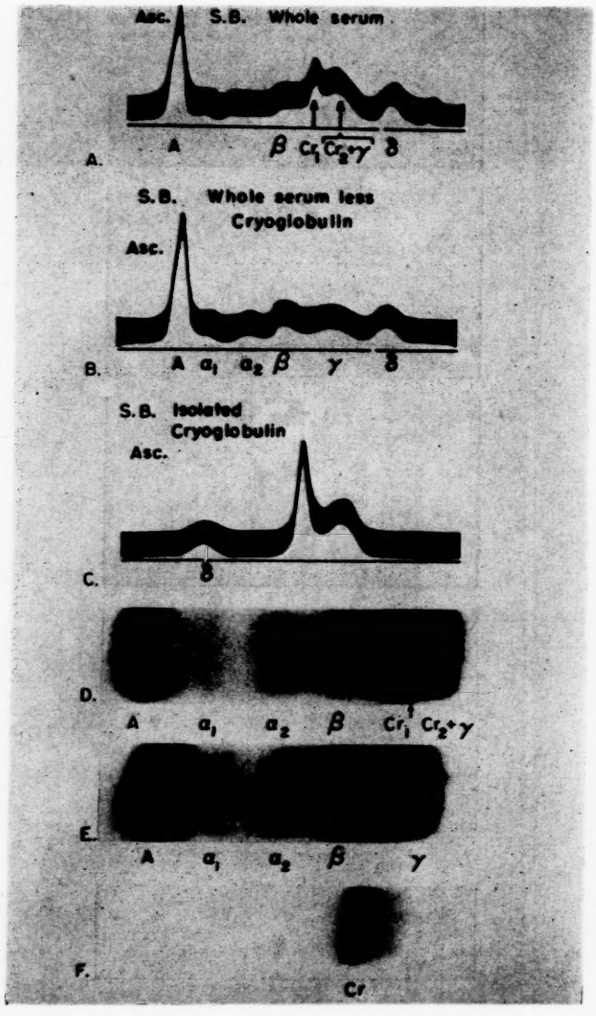


FIG. 2. Case I. Free and paper electrophoretic analyses of serum and cryoglobulin fractions in cryo- and macroglobulinemia; splenomegaly and pancytopenia. A, free electrophoretic pattern, ascending limb; whole serum. B, free electrophoretic pattern, ascending limb; supernatant serum less precipitated cryoglobulin. C, free electrophoretic pattern, ascending limb; isolated washed cryoglobulin. D, paper electrophoretic strip; whole serum. E, paper electrophoretic strip; whole serum less precipitated cryoglobulin. F, paper electrophoretic strip; isolated washed cryoglobulin. Analyses A, B, D, E and F in barbital buffer, pH 8.6, $\mu = 0.1$. Analysis C in phosphate buffer, pH 3.8, $\mu = 0.1$.

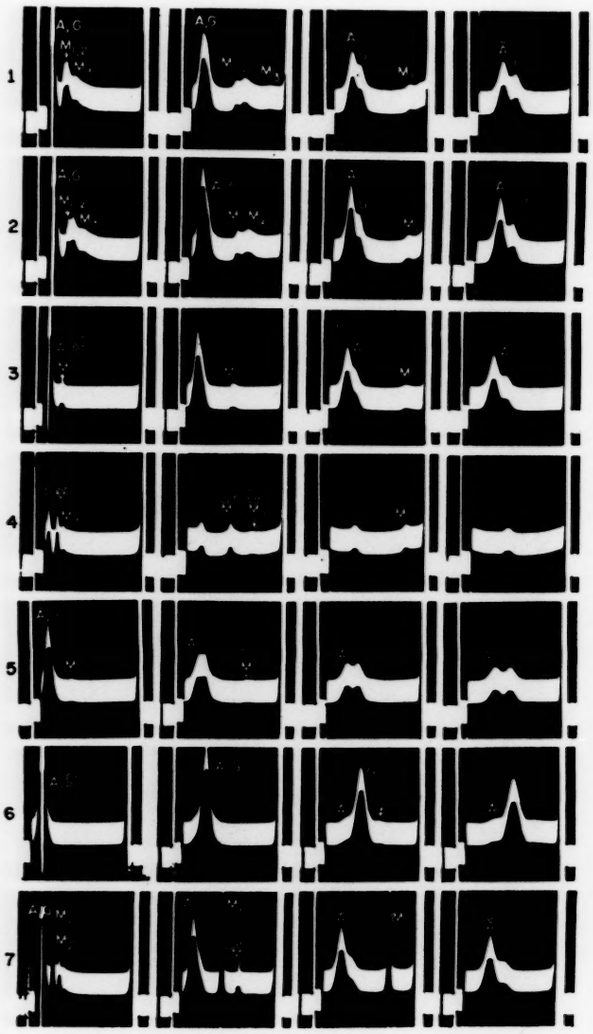


FIG. 3. Ultracentrifugal analyses of serum, cerebrospinal fluid and cryoglobulin fractions in cryo- and macroglobulinemia. (1), Case I. Splenomegaly and pancytopenia; whole serum. (2), same case; cerebrospinal fluid. (3), same case; supernatant serum less precipitated cryoglobulin. (4), same case; isolated washed cryoglobulin. (5), Case III. Multiple myeloma; whole plasma. (6) Same case; heavy cryoglobulin syrupy layer. (7), Case II. Waldenström's macroglobulinemia; whole serum. A = mainly albumin; G = mainly gamma globulin; M = macroglobulins with sedimentation coefficient of 15 S or greater. Analyses performed upon protein dilutions in 0.2 M saline. Analysis (4) in 0.2 M saline – 0.01 M sodium diethylbarbiturate. In normal human serum three components are usually demonstrated: A with sedimentation constant of 4.5 which is chiefly albumin, G with sedimentation constant of 7 which is mainly gamma globulin, and M with sedimentation constant of 15 or greater. The heavy M component gives a very small peak ordinarily and represents proteins of very high molecular weight.

usual 4.5S (A) and 7S (G) peaks, a major 18S macroglobulin peak and other small peaks representing macroglobulins of differing sedimentation coefficients (20S and 27S). (Fig. 3 [1 and 2].) "Cryoglobulin-free" supernate showed a smaller 7S component and only traces of M components. (Fig. 3 [3].) Isolated washed cryoglobulin and a distilled water precipitate showed

7S and 18S components of equal height, with small amounts of other macroglobulins present also. (Fig. 3 [4], Table I.) The Sia screening test for macroglobulins gave strongly positive results in this case.

Macroglobulinemia. Case II: The serum of this patient contained macroglobulins without exhibiting cold precipitation. The macroglobulin resembled a gamma myeloma protein electrophoretically (A and B in Fig. 4); ultracentrifugal analysis (whole serum) revealed a major 15S component and a smaller 20S component, as well as the usual 4.5S (A) and 7S (G) peaks. (Fig. 3 [7], Table I.) Results of the Sia test were negative.

Cryoglobulinemia in Myeloma. Case III: The cryoglobulin of this patient began to precipitate from the oxalated plasma at 32°C. and redissolved at this temperature when warmed from the cold state. Precipitation increased after storage for three days at 4°C., after which time the plasma separated into two layers, a supernatant and a lower syrupy layer containing most of the cryoglobulin. When the plasma was cooled from 4°C. to −5°C. further precipitation in the supernatant occurred, and the lower clear viscous layer formed a stiff opaque coagulum. The concentration of plasma total serum protein was 10.8 gm. per cent, of the supernatant 5.6 gm. per cent, and of the heavy cryoglobulin layer 20.2 gm. per cent. The cryocrit was 33. The cryoglobulin content of whole plasma was approximately 6.7 gm. per cent.

Electrophoretic analyses: Whole plasma showed a typical myeloma gamma globulin component in both paper and free electrophoretic patterns, the cold-precipitating globulin being indistinguishable from myeloma gamma globulin. Analysis of whole plasma in barbital buffer (pH 8.6) at 0°C. was technically not possible, due to precipitation of cryoglobulin. In a 0.2M glycine-0.1N sodium hydroxide buffer (pH 9.67), a single symmetric sharp gamma myeloma peak was present in both limbs. (Fig. 5A.) When the supernate was analyzed in barbital buffer, the unprecipitated gamma globulin was seen as a single discrete gamma myeloma component by paper electrophoresis (Fig. 5C) and in the ascending limb of the free electrophoretic pattern; on the descending limb of the free electrophoretic pattern this peak appeared as a broad low hump, suggesting the presence of additional components. Paper electrophoresis of the syrupy layer (analyzed after prolonged

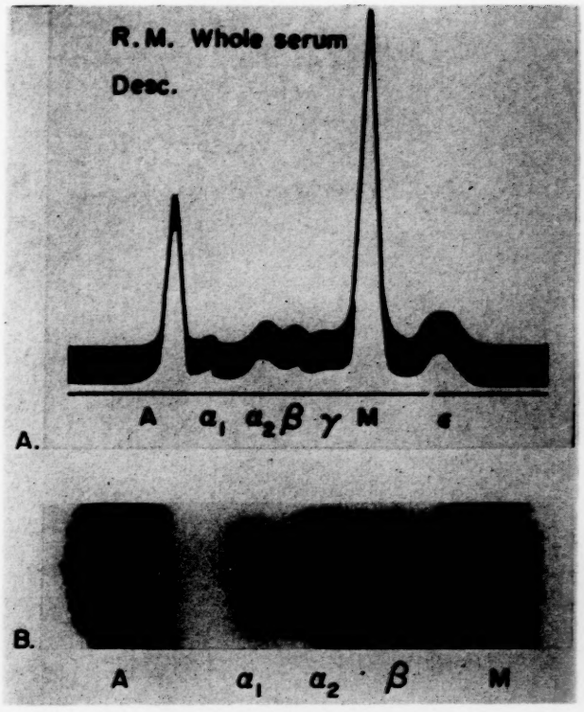


FIG. 4. Case II. Free and paper electrophoretic analyses of serum in macroglobulinemia. Waldenström's macroglobulinemia. A, free electrophoretic pattern of whole serum, descending limb. B, paper electrophoretic pattern of whole serum. Both analyses in barbital buffer, pH 8.6, $\mu = 0.1$.

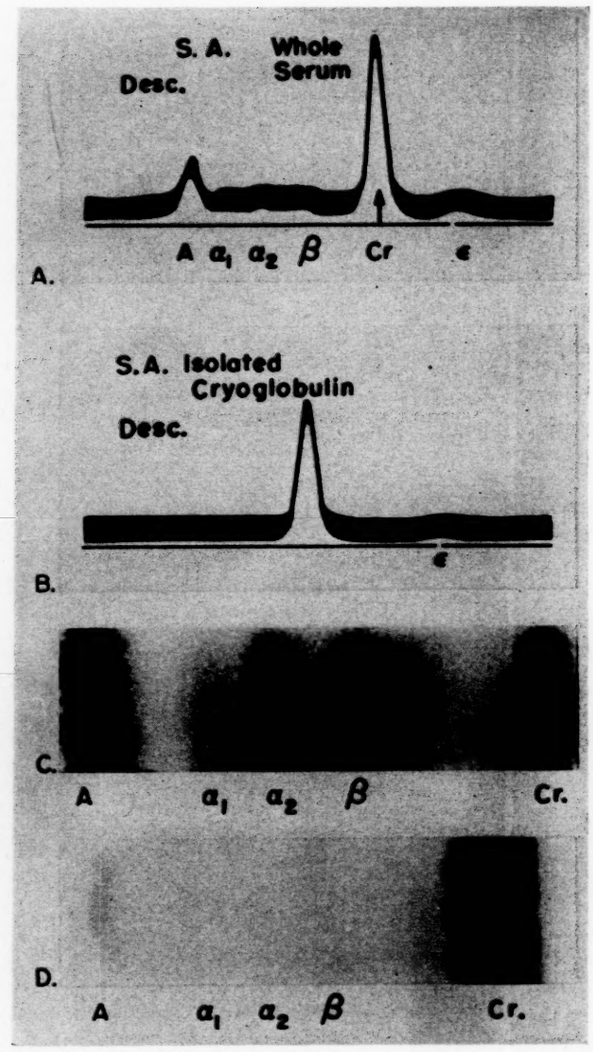
standing) showed the cryoglobulin as a dense band in the gamma globulin position (Fig. 5D); small amounts of albumin and other globulins were also present. Free electrophoresis of isolated cryoglobulin in an acid buffer (acetate pH 4.65, $\mu = 0.1$) gave a single sharp symmetric peak in both limbs.* (Fig. 5B.) Precipitation of gamma globulin (and presumably cryoglobulin) by the method of Wolfson et al.[5] with re-solution in M/20 sodium diethylbarbiturate (pH 9.76) also yielded an electrophoretically homogeneous fraction.

Ultracentrifugal analyses: The supernate showed 4.5S (A) and 7S (G) components in approximately equal concentrations, with only a trace of a 20S component. (Fig. 3[5].) In the lower syrupy layer there was a prominent 7S (G) component, a small 4.5S (A) component, but no trace of any M present. (Fig. 3 [6].)

Both the supernatant and lower syrupy layer gave a strongly positive reaction to the Sia test.

Cryoglobulinemia in Cirrhosis and Myeloma. Case IV: The cryoglobulin of this patient took

* After 140 minutes of migration.



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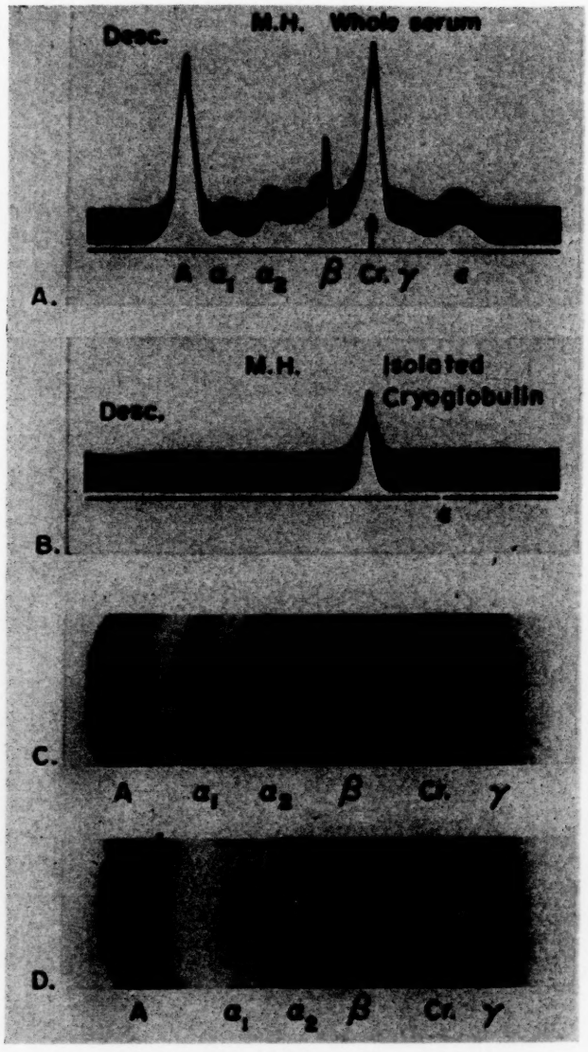

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FIG. 5. Case III. Free and paper electrophoretic analyses of serum and cryoglobulin fraction in cryoglobulinemia. Multiple myeloma. A, free electrophoretic pattern, descending limb; whole plasma. B, free electrophoretic pattern, descending limb; isolated washed cryoglobulin. C, paper electrophoretic strip; supernatant plasma less precipitated cryoglobulin. D, paper electrophoretic strip; heavy cryoglobulin layer. Analysis A in 0.2 M glycine in 0.1 N sodium hydroxide buffer, pH 9.67. Analysis B in acetate buffer, pH 4.65, $\mu = 0.1$. Analyses C and D in barbital buffer, pH 8.6, $\mu = 0.1$.

FIG. 6. Case IV. Free and paper electrophoretic analyses of serum and cryoglobulin fractions in cryoglobulinemia. Multiple myeloma and portal cirrhosis. A, free electrophoretic pattern, descending limb; whole serum. B, free electrophoretic pattern, descending limb; isolated cryoglobulin. C, paper electrophoretic strip; whole serum. D, paper electrophoretic strip; supernatant serum less precipitated cryoglobulin. All analyses in barbital buffer, pH 8.6, $\mu = 0.1$.

the form of a flocculent, noncrystalline precipitate. After standing overnight at 4°C. the serum became a semi-solid gel. The cryocrit measured 20. The Sia test gave negative results.

Electrophoretic analyses: Whole serum showed a spiked gamma$_1$ globulin peak, appearing as a dense discrete band by paper electrophoresis, accompanied with a small amount of gamma$_2$ globulin. (Fig. 6C.) The gamma$_1$ globulin was found to represent 43.1 per cent of the total serum protein (8.36 gm. per cent). This pattern was regarded as typical of multiple myeloma. The isolated saline-washed cryoglobulin had an electrophoretic mobility identical with that of this gamma$_1$ globulin, indicating that this component in the whole serum pattern was actually the cryoglobulin. (Fig. 6B, Table I.) Paper electrophoretic analysis of the supernate at 4°C.

showed this gamma$_1$ band to be still present although in much smaller concentration (Fig. 6D), indicating that not all the cryoglobulin had precipitated at this temperature.

Ultracentrifugal analyses: Whole serum showed 4.5S (A) and 7S (G) components in approximately equal amounts, and a small amount of M component. The isolated cryoglobulin showed a major 7S gamma globulin component. (Table I.)

Idiopathic Cryoglobulinemia. Case V: This cryoglobulin formed a light flocculent precipitate while standing overnight at 4°C. The total amount of cryoglobulin present was small, the cryocrit measuring only 8. Electrophoretically the mobility of this cryoglobulin was intermediate between beta and gamma globulin. The cryoglobulin component was still electrophoretically demonstrable in the supernate following cold precipitation, indicating that some cryoprotein had remained in solution. Macroglobulins were absent. (Table I.) The Sia test gave negative results.

DISCUSSION

The presence in human serum or plasma of large amounts of reversible, cold-precipitating globulins, or cryoglobulins, often associated with diseases displaying bizarre clinical features, has been previously described in nearly fifty publications,* usually based upon single case reports. The subject of cryoglobulinemia was well reviewed by Lerner and Watson in 1947[1] and by Barr, Reader and Wheeler in 1950.[11] Macroglobulins are high molecular weight proteins demonstrable only by ultracentrifugation; the syndrome of macroglobulinemia was first described by Waldenström.[2]

The concentration of cryoglobulin in plasma or serum varies widely from trace amounts up to 10 gm. per cent. (Fig. 1.) In one person the level may be highly variable over the course of the illness (Case I). Amounts greater than 25 mg. per cent correspond to the grade 3 cryoglobulinemia of Lerner, Barnum and Watson,[12] or to the grade 4 "cold fraction" of Wertheimer and Stein.[13] The minimum concentration of cryoprotein required for massive reversible precipitation, coagulation or gelification has been thought to be about 1 gm. per cent;[14] usually the term cryoglobulinemia is reserved for serums of patients showing abnormalities of this magnitude. The separated cold precipitate is not entirely cryoprotein, for even after washing and recooling traces of trapped albumin and other globulins may be demonstrated by electrophoretic analysis.[47] (Fig. 4D, Case III.)

Small amounts of cryoglobulins in serum (i.e. less than 25 mg. per cent) are frequently found in a variety of diseases[11–13,16] but seem of little clinical importance. Massive cryoglobulinemia, however, is not common. Presumably cryoglobulins are not found in normal subjects.[12] Trace amounts of macroglobulins are present in normal serums when examined by the ultracentrifuge. Macromolecular (S19-20) components of normal human serum have been reported by Pedersen[79] to be composed largely of alpha globulins. Oncley, Scatchard and Brown[80] reported lipid-free beta$_1$ globulins with an S constant of 20 in normal human plasma. Sholtan[15] states that the S20 component of normal human serum migrates as a beta$_1$ globulin electrophoretically. Pathologic macroglobulin molecules have the electrophoretic mobility of beta or gamma globulins.[4] It may be pointed out that properdin, a recently discovered globulin which is of key importance in natural immunity, has a sedimentation constant of 27S and thus is a macroglobulin.[81] The term macroglobulinemia is reserved for serums containing more than 5 per cent of components sedimenting with Svedberg constants greater than 15.*

Cryoglobulinemia is most often associated with multiple myeloma, although cryoglobulins are not commonly reported in this disease. The diagnosis of myelomatosis should therefore be considered in all instances of cryoglobulinemia. In several reported cases of cryoglobulinemia[18–20] certain clinical and autopsy findings (particularly plasmacytosis of the bone marrow) suggested multiple myeloma, although this diagnosis was not conclusively established. Cryoglobulins even in massive concentrations are not pathognomonic of any single disease, however, and may be found in a variety of chronic disorders associated with hyperglobulinemia, including chronic lymphatic leukemia,[21–23] lymphosarcoma,[24] subacute bacterial endocarditis,[16] kala-azar,[13,25] malaria,[13] polyarteritis nodosa,[26,77] rheumatoid arthritis,[27] lupus erythematosus[9] and other chronic inflammatory conditions, all of which show involvement of the reticuloendothelial system. Occasionally, there may be no

* See Accessory Table and References pages 582 to 587, inclusive.

* For normal human serums, less than 3 per cent of proteins have S constants of 15 or greater.[4,15,97]

apparent underlying diseases (Case v).[4,28] Cryoglobulinemia in dogs infected with kala-azar has also been demonstrated.[78]

The true place of macroglobulinemia in nosology must await future studies. In some cases abnormal macroglobulins appear in the absence of any well definable disease process. However, reticuloendothelial proliferation of cells intermediate between lymphocytes and plasma cells has been frequently but not invariably observed (as in Case II). Such cases have been classified as Macroglobulinemia of Waldenström.[4,29–31] Instances of significant macroglobulinemia in cancer[32] and congenital syphilis[33] have been described. Small increases in macroglobulins are frequently observed in the nephrotic syndrome and in cirrhosis.*[34,35] It should be emphasized that serums of multiple myeloma have seldom been reported to show abnormal high molecular weight components.[4,53,82] On the other hand macroglobulinemia patients who never show focal bone lesions have exhibited Bence-Jones proteinuria[36] and also paramyloidosis such as is seen in myelomatosis.[31,37] It has been proposed that macroglobulinemia is a variant of myelomatosis.[17,31] For clinical purposes, however, the two entities of multiple myeloma and Waldenström's macroglobulinemia should be considered different.

Cryoglobulinemia is often but not invariably characterized by a definite clinical syndrome. The primary clinical feature is intolerance to cold, as manifested by Raynaud's phenomenon, purpura, urticaria, ulceration of the skin, peripheral gangrene and retinal vascular stasis.[1,4,11,14] Increased viscosity of the serum, rouleaux formation, pseudo-autoagglutination of the red cells and an elevated erythrocyte sedimentation rate are common in multiple myeloma (and other hyperglobulinemias) without cryoglobulins as well as in states of cryoglobulinemia.[11] It appears likely that the following three factors determine the production of symptoms in these cases: (1) the amount of cryoglobulin present; (2) its solubility characteristics in relation to temperature; and (3) the interaction of cryoglobulin with other plasma proteins. Symptoms may be present when the cryoglobulin concentration is minimal. Of the patients presented in this paper only in Case v in which the serum contained the least amount of cryoglobulin, were there symptoms which could be considered directly referable to cryoglobulinemia. Similarly, the first patient of Barr, Reader and Wheeler,[11] with a serum cryoglobulin concentration of merely 22 mg. per cent, had the classic symptoms usually attributed to cryoglobulinemia; (it seems doubtful that such low concentrations of cryoglobulins are causally related to cold purpura and Raynaud's phenomenon). Symptoms are due largely to precipitation of cryoprotein in peripheral blood vessels. Cold agglutination of red cells with vascular obstruction may also be a factor.[21,39] The bleeding tendency may depend upon intravascular precipitations with secondary capillary damage; defects in platelet function may also contribute.[40]

Whether or not precipitation of cryoglobulin commonly occurs in deep vessels is uncertain. Pulmonary arteriolar obstruction with pulmonary hypertension has been attributed to cryoglobulins.[20] Several patients died from uremia, suggesting the possibility of renal vascular precipitation of cryoprotein. Trace amounts of cryoglobulins were found in 40 per cent of patients with myocardial ischemia; the significance of this was considered doubtful.[41] Multiple visceral intravascular thromboses occurred in the case described by Cugudda.[42] Cerebral purpura associated with cryoglobulinemia has been reported.[43]

Patients with Waldenström's syndrome of macroglobulinemia[3,4,29–31,44,84] characteristically suffer from lassitude, dyspnea and mucosal bleeding. Examination reveals pallor, edema, slight hepatosplenomegaly and painless mild enlargement of lymph nodes. Epistaxis and mucosal bleeding are frequent. Intracerebral bleeding occurred in Case I of this series. Usually there is a markedly elevated sedimentation rate, anemia and lymphocytosis. The bone marrow characteristically shows large numbers of small atypical "lymphocytic" cells with protoplasmic shedding. Isolated leukopenia, thrombocytopenia and acquired hemolytic anemia[74] have been observed; pancytopenia, as in our Case I, may occur.[36] The serum viscosity is greater than normal and increases further with reduction of temperature, gel formation often occurring.[4,84]

According to Waldenström[5,9] a positive reaction to the Sia test suggests macroglobulinemia. This test gave positive results in our Case I, in which large amounts of macroglobulins were

* Recent immunologic studies by Kanzow and coworkers[97] appear to establish further Waldenström's macroglobulinemia as a definite disease entity in which the macroglobulins are distinctly different from those appearing in various other diseases.

PATHOPHYSIOLOGY AND SYMPTOM PRODUCTION IN CRYO- AND MACROGLOBULINEMIA

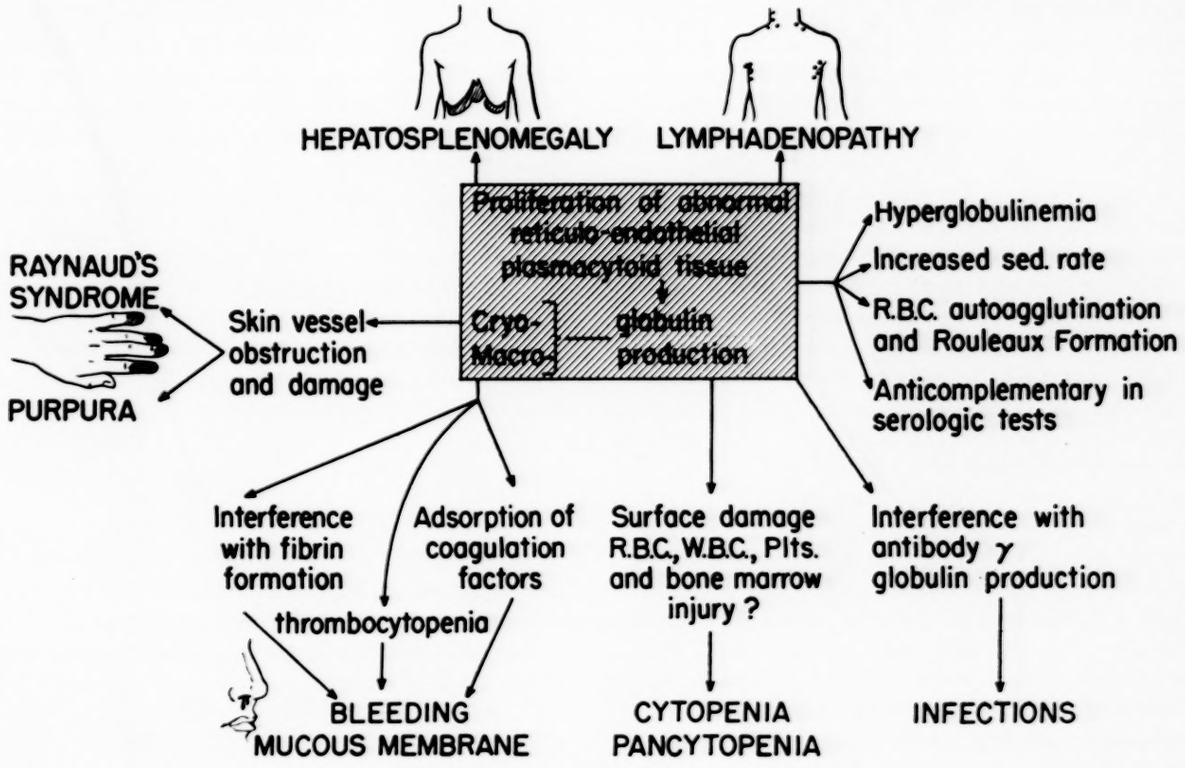

FIG. 7.

present; however, in Case III, in which no increased amounts of macroglobulins were present, a positive reaction was also found. We have seen other patients with hyperglobulinemia whose serum gave positive reactions to the Sia test in the absence of macroglobulinemia. Conversely, Case II, with gross macroglobulinemia, gave a negative reaction to the Sia water test. The lack of reliability of this screening test has also been reported by others.[4,31,47,76,83,96]

Our concept of the pathologic physiology of cryo- and macroglobulinemia is illustrated diagrammatically in Figure 7. Infections are common in both conditions and are probably related to failure of antibody formation resulting from competitive use of amino acids for synthesis of abnormal protein. Such a mechanism has been demonstrated in multiple myeloma.[45,46] Bleeding tendencies are presumably explained by interference with fibrin formation, adsorption of coagulation factors upon the macroglobulins, thrombopenia, and direct damage of capillary walls from cooling.[56,89,90,94,98] In a case of cryo- and macroglobulinemia Braunsteiner and coworkers[40] demonstrated, by means of electron microscopy, inhibition of pseudopod formation and defective adhesiveness of platelets. Sjögren's syndrome (dryness of mucous membranes in eyes, nose, mouth and vagina[91]) has been reported in several cases of cryo- and macroglobulinemia[30,37] and was a striking feature of the clinical symptoms of Case V. It is conceivable that these abnormal proteins may at times interfere with normal glandular secretions.

Physicochemical properties: The physical properties of cryoglobulins and macroglobulins are highly variable. It is likely that these proteins represent broad groups of abnormal globulins which cannot be consistently identified with any particular plasma component. In addition to cryoglobulins, cryofibrinogens also exist. We have already observed a patient with autoimmune hemolytic disease and multiple thrombotic episodes whose plasma contained a heparin-precipitable cryofibrinogen.* Similar cryofi-

* This heparin-precipitable cryoprotein is closely associated with fibrinogen but has not been proved to be identical with fibrinogen.

brinogens have been studied by Thomas et al.[48] in patients with acute rheumatic fever and in rabbits injected with bacterial toxins; lesser amounts were found in plasma of normal humans. Yet another type of cryofibrinogen from oxalated plasma has been recently described by Korse and Kratochril[49] in a patient with multiple thromboses and pulmonary neoplasm. Whereas the phenomenon described by Thomas appears to require the chemical interaction of heparin, the majority of cryoproteins found in serum or plasma undergo precipitation spontaneously, even after repeated washing in saline solution. These molecular characteristics would seem to reflect an abnormality of chemical structure or spatial relationships which at low temperature results in insolubility, and perhaps also molecular polymerization.[17,50,51]

Most commonly, cryoglobulins appear as a dense flocculent precipitate. Cryoglobulin precipitates may be wholly or partially crystalline. Others may form a solid coagulum or gel on cooling, which "thaws" on warming. Occasionally, the gel will not completely redissolve on heating.[14] In some instances a precipitate may form initially on cooling, which may be followed on further cooling or storage at low temperature by complete coagulation of the serum. Cryoglobulins frequently sediment out as a lower syrupy layer (Case III).[57,72,85–87]

The concentration of cryoglobulin in serum is usually estimated from the difference in serum total protein before and after cold precipitation. Quantitative estimation of the concentration of cryoglobulin can hardly be precise, as precipitation depends directly upon temperature and the cryoprotein may take several days for maximal precipitation at a given temperature.[41] Complete precipitation even at 0°C. probably never occurs. An approximate estimate of the amount present can be obtained by the protein-cryocrit as described in this paper, although this measures the relative volume of the precipitated cryoglobulin rather than its concentration in grams per cent. The amount of macroglobulins can only be estimated from planimetry of the ultracentrifugal diagram.

The electrophoretic and ultracentrifugal data presented here further emphasize the lack of uniformity in the physicochemical characteristics of cryo- and macroglobulins.[51–54,100] There have been several reports of an abnormal plasma protein being both a cryo- and macroglobulin (Case I).[2,19,24,51,54,55,57,75,76] Both macro- and cryoglobulins usually migrate electrophoretically near the fibrinogen area between β and γ globulin. The mobility of cryoglobulins may range from that of α_2 globulin[42] to that of slow γ_2 globulins (Case III).[22] Myeloma cryoglobulins are usually identical with the abnormal myeloma proteins (Cases III and IV) and show the same physical properties. Macroglobulin peaks in the electrophoretic diagram are sharp and cannot be differentiated from the peaks of myeloma proteins. (Fig. 4.) Thus ultracentrifugation is necessary for the recognition of macroglobulinemia.*

Macroglobulins frequently sediment as multiple components (Cases I and II). This phenomenon has been observed previously.[19,24,29,40,51,57] Pedersen originally suggested that macromolecules may result from association-dissociation reactions.[50] Petermann and Braunsteiner[51] demonstrated that such reactions in human serum depend upon physical factors such as pH, ionic strength, protein concentration and temperature.

Of particular interest was the non-homogeneity of the isolated macroglobulin complex of Case I. This showed two separate components electrophoretically and ultracentrifugally which were consistently present in all analyses undertaken. The sedimentation coefficients of these two components were 7S and 18S, suggesting that this cryoglobulin was present as a molecular complex composed of gamma globulin and macroglobulin. A similar type of cryoglobulin complex has been previously described.[22] It is possible that the macroglobulin element of this complex in our Case I represented a molecular aggregate of the gamma globulin rather than a separate protein species. Partial denaturation of the cryoglobulin, due to pH changes in the serum on standing, might be considered as a possible explanation of the double component demonstrated electrophoretically.[56]

Studies of the amino acid composition of macroglobulins have shown variations from patient to patient.[36] As compared with normal beta and gamma globulins, an abnormally low lysine content was found in four cases studied by Pernis et al.[36] In addition, a low proline content was also found in three of these four, as well as in a patient investigated by Mandema.[57]

* Vogler et al.[54] have reported that severe macroglobulinemia may be detected without ultracentrifugal analysis by combined electrophoretic and diffusion measurements.

Hydroxyproline, which is not found in normal gamma globulin, was present in a case of gamma cryomacroglobulinemia.[57] Greater than normal quantities of lysine and histidine were found in the gamma cryoglobulin analyzed by Dustin and Leonis.[56] Methionine content, which is very low in concentration in Bence-Jones proteins, was the same as in normal gamma globulin in all these cases; however, the methionine content was found extremely low in the cryoglobulin analyzed by Harvier et al.[92] Grümer and his coworkers[93] demonstrated in three cases of macroglobulinemia a lower content of tryptophane and tyrosine than is present in normal gamma globulins. In spite of those few differences the general amino acid pattern of these abnormal plasma proteins has been found to resemble rather closely that of beta or gamma globulins.[19,36,37,56,57,95]

Origin and Immunologic Specificity. Ehrich[58,59] has reviewed the evidence for the concept that plasma cells are the source of both immune globulins and abnormal plasma proteins; Barr,[4] Bianchi[17] and their respective coworkers offer some evidence from their own cases that plasma cells participate in the synthesis of cryoglobulin. There is certainly an increase in plasma cells in the bone marrow in many reported cases of cryoglobulinemia, as well as in other varieties of hyperglobulinemia. In some instances of cryoglobulinemia, however, there has been no increase in the bone marrow.[21] In the case studied by Abrams, Cohen and Meyer[24] the cryoglobulin isolated from lymphosarcoma tissue appeared identical with that present in the peripheral blood. In Waldenström's macroglobulinemia, lymphocyte-like cells derived from reticuloendothelial tissue, which have been considered intermediate forms between plasma cells and lymphocytes, presumably are involved in the elaboration of the macroglobulin. However, such cells have not been consistently demonstrated in all cases of macroglobulinemia, as in our Case 1. Thus it seems that although plasma cells are the usual source of cryoglobulins, and atypical lymphocytic cells may manufacture macroglobulins, other more primitive or malignant forms of these cell types can also produce these abnormal plasma proteins.

It is important to know whether these abnormal protein species represent increases of normally occurring protein molecules (dysproteinemia) or whether their presence actually denotes aberrant protein synthesis (paraproteinemia). Cryoglobulin antisera have been produced[21,75] which showed cross reactions with normal whole serum and gamma globulin but not with albumin. These results would not indicate different configuration of cryoglobulin molecules. However, extensive immunologic work with macroglobulins has demonstrated these abnormal proteins to be of immunologic specificity differing from normal serum proteins.*[19,60,61,97] Thus the serums of fourteen of sixteen patients gave a positive reaction to the precipitin test against rabbit antimacroglobulin serums which had been absorbed with normal human serum;[60,61] it is of further interest that although this whole group shared certain antigenic reactions, macroglobulins from several patients were individually specific and immunologically different from other macroglobulins.[60,61] Similar findings have recently been reported for certain myeloma proteins.†[62,63] These immunologic findings support the concept that macroglobulins and myeloma proteins actually represent truly abnormal molecular species. This concept has recently been questioned, however, from the results of other immunologic studies.[65,99,100]

The molecular weight of various cryoglobulins has ranged from that of gamma globulins (150,000–170,000) to over one million. These proteins have been demonstrated in nearly all fluid compartments of the body through which plasma proteins usually circulate, including that of cerebrospinal fluid (Case 1).[4,88] The occurrence of very high molecular weight macroglobulins in cerebrospinal fluid (our Case 1) and in the fluid of cantharides blisters[67] is of special interest; presumably, alteration of membrane permeability explains the entry of plasma macroglobulins into blister fluid and their occurrence in cerebrospinal fluid following cerebrovascular thrombosis.

Differential Diagnosis and Prognosis. The diagnosis of cryoglobulinemia offers no particular difficulty. Since some cryoglobulins have a wide thermal amplitude, blood for these studies should be drawn into warm syringes and separation of serum or plasma carried out at 37°C. if

* In addition, Kanzow and his collaborators[97] have reported immunologic distinction between the macroglobulins of Waldenström's disease and the macroglobulins appearing in states of nephrosis and cirrhosis.

† N-terminal amino acid groups of myeloma proteins and cryoglobulins have also been found individually specific and different from normal globulins.[64,99,100]

possible. Three cryoglobulins showing some precipitation even at body temperature have been described.[18,27,68] Cold precipitation *reversible on warming* is characteristic and establishes the diagnosis of cryoglobulinemia. Macroglobulinemia is diagnosed with more difficulty. As pointed out, the simple Sia (Brahmachari) water dilution test is misleading and may give false positive and negative results. Immunologic diagnosis with antimacroglobulin serum has been successful in almost all cases studied,[60,61,97] but ultracentrifugal analysis alone is finally conclusive. The demonstration of single or multiple high molecular weight proteins sedimenting with Svedberg constants of more than 15S and in significant concentration (greater than 5 per cent) is diagnostic of macroglobulinemia.

The differential diagnosis of cryoglobulinemia should include cryofibrinogens, heparin-precipitable cryofibrinogens and cold agglutinins. If plasma with cold agglutinins is placed in a sedimentation tube at 4°C., sedimentation will be accelerated due to agglutination of erythrocytes, while precipitation or jelling of cryoglobulins in the cold will slow sedimentation.[4] The effect of cryoglobulin on the sedimentation rate at room temperature is dependent upon its individual thermal characteristics; when readily soluble, an increased sedimentation rate is usual.[38] Although most peripheral vascular diseases are not caused by cryoglobulins, the simple cryoglobulin screening tests should be performed on all such patients and others with dependent purpura. If cryoglobulins are discovered, multiple myeloma should be carefully searched for by bone marrow puncture, serum and urine electrophoresis[69] and roentgenologic skeletal survey.

Macroglobulinemia should be distinguished from purpura hyperglobulinemica, a condition which also has been defined by Waldenström.[3,70] Purpura hyperglobulinemica[71] is characterized by dependent purpura associated with hypergammaglobulinemia. The characteristic electrophoretic gamma globulin peak in this syndrome is a broad hump in contrast to the sharp peak of macroglobulinemia. Electrophoretically, myelomatosis cannot be differentiated from macroglobulinemia. (Fig. 3.) Ultracentrifugally, however, high molecular components are not found in multiple myeloma.[4] Because of bone marrow and lymph node infiltration with small atypical lymphocytes, as well as lymphadenopathy and hepatosplenomegaly, chronic lymphatic leukemia and lymphosarcoma may be difficult to differentiate from macroglobulinemia. It appears probable that many atypical cases of myelomatosis and lymphatic leukemia[17,18,23,72,73,83] actually represent instances of macroglobulinemia.

Since cytopenia and pancytopenia are not uncommon in macroglobulinemia, it appears desirable to include ultracentrifugal examination of serum in the investigation of such ill defined hematologic syndromes. In view of the ease with which patients with acquired hemolytic anemia produce abnormal plasma proteins, it is interesting that macroglobulins have been observed in this condition.[74] A further search for macroglobulinemia among such cases appears desirable.

The prognosis of cryoglobulinemia depends upon the nature of the underlying disease process. Although most commonly associated with multiple myeloma, it becomes apparent that with the recently developed ability to make an earlier diagnosis of myelomatosis some such patients may live for many years without difficulties. Macroglobulinemia similarly is compatible with survival for several years. If death occurs in either condition it is usually due to severe anemia, failure to combat infections, or hemorrhagic diathesis. Treatment of macroglobulinemia has been unsatisfactory; ACTH and cortisone have not been successful, and splenectomy in our case was of no value.

SUMMARY

Five patients with varying degrees of cryo- and macroglobulinemia have been studied and the physicochemical characteristics of these abnormal proteins investigated.

1. Macroglobulins are giant protein molecules with a molecular weight of over one million. Cryoglobulins are proteins which precipitate on cooling but redissolve on warming to body temperature. Occasionally, an abnormal plasma protein may be both a cryo- and macroglobulin. These abnormal proteins are probably elaborated by the reticuloendothelial-plasmacyte system.

2. The electrophoretic mobility of cryo- and macroglobulins ranged between that of beta and gamma globulins. Electrophoretic patterns of severe macroglobulinemia are indistinguishable from those of myelomatosis.

3. Ultracentrifugal analysis is required for the

detection of macroglobulins (S constant greater than 15). Multiple macroglobulin components with differing sedimentation constants were found. Macroglobulinemia is not present in myelomatosis.

4. The physicochemical characteristics of these proteins were striking in their variability.

5. A "protein-cryocrit" determination was devised for rapid rough quantitation of cryoglobulins.

6. The water-dilution screening test (Sia or Brahmachari test) was found unreliable for detecting macroglobulins.

7. Clinically, cryoglobulins may be causally related to Raynaud's syndrome and cold purpura. However, no regular relationship could be found between the amount of cryoglobulins and the presence or severity of such symptoms.

8. Cryo- and macroglobulinemia is not specific for any disease entity but cryoglobulins are most frequently seen in myelomatosis. Marked macroglobulinemia is frequently associated with a definite clinical syndrome classified at present as "Macroglobulinemia of Waldenström" with dyspnea, mucous membrane bleeding and bone marrow infiltration of small atypical lymphocytic cells. Immunologically, these macroglobulins may be distinct.

9. The literature of cryo- and macroglobulinemia is comprehensively surveyed and discussed. The pathologic physiology of cryo- and macroglobulinemia is portrayed diagrammatically.

REFERENCES

1. Lerner, A. B. and Watson, C. J. Studies of cryoglobulins. I. Unusual purpura associated with the presence of a high concentration of cryoglobulin (cold precipitable serum globulin). *Am. J. M. Sc.*, 214: 410, 1947.
2. Waldenström, J. Incipient myelomatosis or "essential" hyperglobulinemia with fibrinogenopenia—a new syndrome? *Acta med. Scandinav.*, 117: 216, 1944.
3. Waldenström, J. Zwei interessante Syndrome mit Hyperglobulinämie (Purpura hyperglobulinämica und macroglobulinämica). *Schweiz. med. Wchnschr.*, 78: 927, 1948.
4. Waldenström, J. Abnormal proteins in myeloma. In: Advances in Internal Medicine, vol. 5, p. 398. Edited by Dock, W. and Snapper, I. Chicago, 1952. The Year Book Publishers, Inc.
5. Wolfson, W. Q., Cohn, C., Calvary, E. and Ichiba, F. Studies in serum proteins; rapid procedure for estimation of total protein, true albumin, total globulin, alpha globulin, beta globulin and gamma globulin in 1.0 ml. of serum. *Am. J. Clin. Path.*, 18: 723, 1948.
6. Volwiler, W., Goldsworthy, P. D., MacMartin, M. P., Wood, P. A., Mackay, I. R., and Fremont-Smith, K. Biosynthetic determination with radioactive sulphur of turn-over rates of various plasma proteins in normal and cirrhotic man. *J. Clin. Investigation*, 34: 1126, 1955.
7. (*a*) Sia, R. H. P. and Wu, H. Serum globulin in kala-azar. *China M. J.*, 35: 527, 1921. (*b*) Sia, R. H. P. Simple method for estimating quantitative differences in globulin precipitation test in kala-azar. *China M. J.*, 38: 35, 1924.
8. (*a*) Brahmachari, U. N. On the presence of an easily precipitable anti-complementary globulin-like substance in human serum and its importance in the diagnosis of kala-azar. *Indian M. Gaz.*, 52: 429, 1917. (*b*) Brahmachari, U. N. and Sen, P. B. Globulin opacity test for kala-azar. *Indian M. Gaz.*, 58: 295, 1923.
9. Waldenström, J. Pathological globulins and protein synthesis. *Exper. Med. & Surg.*, 12: 187, 1954.
10. Mackay, I. R., Volwiler, W. and Goldsworthy, P. D. Paper electrophoresis of serum proteins: Photometric quantitation and comparison with free electrophoresis. *J. Clin. Investigation*, 33: 855, 1954.
11. Barr, D. P., Reader, G. G. and Wheeler, C. H. Cryoglobulinemia. I. Report of two cases with discussion of clinical manifestations, incidence and significance. *Ann. Int. Med.*, 32: 6, 1950.
12. Lerner, A. B., Barnum, C. P. and Watson, C. J. Studies of cryoglobulins. II. The spontaneous precipitation of protein from serum at 5°C. in various disease states. *Am. J. M. Sc.*, 214: 416, 1947.
13. Wertheimer, E. and Stein, L. The cold susceptible globulin fraction of pathological sera. *J. Lab. & Clin. Med.*, 29: 1082, 1944.
14. Brauman, J., Gregoire, F., Lambert, P. P., Kleyntjens, F. and Danis, P. Un cas de myelome à cryoglobuline. Étude critique, chimique et physiologique. *Acta clin. Belg.*, 8: 333, 1953.
15. Scholtan, W. Klinische Ultrazentrifugen-studien. I. Mitteilung. Ultrazentrifugen Diagram des normalen menschlichen Serums. *Ztschr. f. d. ges. exper. Med.*, 121: 574, 1953.
16. Dreyfuss, F. and Librach, G. Cold precipitable serum globulins ("cold fractions," "cryoglobulins") in subacute bacterial endocarditis. *J. Lab. & Clin. Med.*, 40: 489, 1952.
17. Bianchi, V., Giampalmo, A. and Marmont, A. Contributo alla conoscenza della gelificazione plasmatica "a frigore" e della plasmocitosi aleucemica con emogelificazione (gel-plasmocitomatosi). *Minerva med.*, 40: 101, 1949.
18. Hansen, P. F. and Faber, M. Raynaud's syndrome originating from reversible precipitation of protein. *Acta med. Scandinav.*, 129: 81, 1947.
19. McFarlane, A. S., Dovey, A., Slack, H. G. B. and Papastamatis, S. C. An unusual case of hyperglobulinaemia. *J. Path. & Bact.*, 64: 335, 1952.
20. Muirhead, E. E., Montgomery, P. O. and Gordon, C. E. Thromboembolic pulmonary vascular sclerosis: report of a case following pregnancy and of a case associated with cryoglobulinemia. *Arch. Int. Med.*, 89: 41, 1952.
21. Schwartz, T. B. and Jager, B. V. Cryoglobulin-

emia and Raynaud's syndrome in a case of chronic lymphatic leukemia. *Cancer*, 2: 319, 1949.

22. Craig, A. B., Waterhouse, C. and Young, L. E. Autoimmune hemolytic disease and cryoglobulinemia associated with chronic lymphatic leukemia. *Am. J. Med.*, 13: 793, 1952.
23. Rundles, R. W., Coonrad, E. V. and Arends, T. Serum proteins in leukemia. *Am. J. Med.*, 16: 842, 1954.
24. Abrams, A., Cohen, P. P. and Meyer, O. O. The physical properties of a cryoglobulin obtained from lymph nodes and serum of a case of lymphosarcoma. *J. Biol. Chem.*, 181: 237, 1949.
25. Most, H. and Lavietes, P. H. Kala azar in American military personnel. *Medicine*, 26: 221, 1947.
26. Shapiro, B. and Wertheimer, E. Spontaneous crystallization of a protein from pathological human serum. *Brit. J. Exper. Path.*, 27: 225, 1946.
27. Holmberg, C. G. and Grönwall, A. Ein neues krystalinisches Serumglobulin. *Ztschr. f. physiol. Chem.*, 273: 199, 1942.
28. Steinhardt, M. J. and Fisher, G. S. Cold urticaria and purpura as allergic aspects of cryoglobulinemia. *J. Allergy*, 24: 335, 1953.
29. Kanzow, U. Die Makroglobulinämie Waldenström. *Klin. Wchnschr.*, 32: 154, 1954.
30. Tischendorf, W. and Hartmann, R. F. Makroglobulinaemie (Waldenström) mit gleichzeitiger Hyperplasie der Gewebsmastzellen. *Acta haemat.*, 4: 374, 1950.
31. Mandema, E. Macroglobulinaemia Waldenström. *Nederl. tijdschr. v. geneesk.*, 98: 2109, 1954.
32. Schaub, F. Gleichzeitiges Vorkommen von Makroglobulinämie Waldenström und von malignen Tumoren. *Schweiz. med. Wchnschr.*, 83: 1256, 1953.
33. Willi, H., Koller, F. and Raaflaub, I. Symptomatische Macroglobulinämie bei Lues congenita. *Acta haemat.*, 11: 316, 1954.
34. Jahnke, K. and Scholtan, W. Klinische ultrazentrifugen Untersuchungen. II. Mitteilung. Pathologische Veränderungen im Serum-ultrazentrifugendiagramm. *Ztschr. f. d. ges. exper. Med.*, 122: 39, 1953.
35. Eriksen, N. Personal observations.
36. Pernis, B., Wuhrmann, F. and Wunderly, C. Aminoacid composition of macroglobulins in four cases of macroglobulinemia. *Acta haemat.*, 2: 309, 1954.
37. Wunderly, C. and Wuhrmann, F. Zu der Bausteinanalyse atypischer Plasmaproteine. *Exper. Med. & Surg.*, 12: 171, 1954.
38. Luckey, E. H., Russ, E. and Barr, D. P. Cryoglobulinemia. III. Influence of a cryoglobulin on the suspension stability and sedimentation rate of erythrocytes. *J. Lab. & Clin. Med.*, 37: 253, 1951.
39. Rörvik, K. Cryoglobulinemia: a survey and a case report. *Acta med. Scandinav.*, 137: 390, 1950.
40. Braunsteiner, H., Falkner, R., Neumayer, A. and Pakesch, F. Macromolekulare Kryoglobulinämie. *Klin. Wchnschr.*, 32: 722, 1954.
41. James, T. J. and Drake, E. H. Cryoglobulins in coronary artery disease. *New England J. Med.*, 249: 601, 1953.
42. Cugudda, E. Plasmocitoma con crioglobulinemia e trombosi arteriose e venose multiple. *Minerva med.*, 2: 205, 1952.
43. Marshall, R. J. and Malone, R. G. S. Cryoglobulinaemia with cerebral purpura. *Brit. M. J.*, 11: 279, 1954.
44. Wilde, H. and Hitzelberger, A. L. Macroglobulinemia: clinical features and differential diagnosis. *Blood*, 9: 875, 1954.
45. Zinneman, H. H. and Hall, W. H. Recurrent pneumonia in multiple myeloma and some observations on immunologic responses. *Ann. Int. Med.*, 41: 1152, 1954.
46. Lawson, H. A., Stuart, C. A., Paull, A. M., Phillips, A. M. and Phillips, R. W. Observations on the antibody content of the blood in patients with multiple myeloma. *New England J. Med.*, 252: 13, 1955.
47. Feiereis, H. and Schnert, H-E. Seltene Form einer Paraproteinose. *Klin. Wchnschr.*, 32: 998, 1954.
48. Thomas, L., Smith, R. T. and Von Korff, R. Cold-precipitation by heparin of a protein in rabbit and human plasma. *Proc. Soc. Exper. Biol. & Med.*, 86: 813, 1954.
49. Korse, D. R., and Kratochril, C. H. Identification of a cryofibrinogen in a case of lung neoplasm associated with thrombophlebitis migrans. *Clin. Research Proc.*, 3: 20, 1955.
50. Pedersen, K. O. Size relationship among similar proteins. Association and dissociation reactions of protein units. *Cold Spring Harbor Symp. Quant. Biol.*, 14: 140, 1949.
51. Petermann, M. L. and Braunsteiner, H. A cryoglobulin of high sedimentation rate (macroglobulin) from human serum. *Arch. Biochem. & Biophys.*, 53: 491, 1954.
52. Olhagen, B. Spontaneous precipitation of crystalline globulin in myeloma serums. *Acta med. Scandinav. (supp.)*, 239: 86, 1950.
53. Putnam, F. W. and Udin, B. Proteins in multiple myeloma. I. Physicochemical study of serum proteins. *J. Biol. Chem.*, 202: 727, 1953.
54. Vogler, K., Oberhänsli, W. and Kopfler, M. Zum Nachweis der Paraproteine bei Makroglobulinämien. *Helvet. med. Acta*, 22: 146, 1955.
55. Gard, S., Heller, L. and Malmros, H. Electron microscopy of euglobulin from a case of hyperglobulinemia. *Proc. Soc. Exper. Biol. & Med.*, 79: 328, 1952.
56. Dustin, J. P. and Léonis, J. A propos d'une cryoglobuline. *Exper. Med. & Surg.*, 12: 192, 1954.
57. Mandema, E., Van der Schaaf, P. C. and Huisman, T. H. J. Investigations on the amino acid composition of a macroglobulin and a cryoglobulin. *J. Lab. & Clin. Med.*, 45: 261, 1955.
58. Ehrich, W. E. Cellular sources of antibodies. In: Blood Cells and Plasma Proteins, p. 187. Edited by Tullis, J. L. New York, 1953. Academic Press, Inc.
59. Ehrich, W. E. Die cellulären Bildungsttäten der Antikörper. *Klin. Wchnschr.*, 33: 315, 1955.
60. Habich, H. Zur Antigenanalyse der Paraproteine bei Makroglobulinämien. *Schweiz. med. Wchnschr.*, 83: 1253, 1953.
61. Habich, H. and Hässig, A. Essai d'analyse antigénique des paraprotides dans la macroglobulinémie de Waldenström. *Vox Sanguinis*, 3: 99, 1953.
62. Slater, R. J., Ward, S. M. and Kunkel, H. G. Immunological relationships among the myeloma proteins. *J. Exper. Med.*, 101: 85, 1955.

63. Wuhrmann, F. H., Wunderly, C. and Hässig, A. Immunological investigations in ten cases of plasmacytoma. *Brit. J. Exper. Path.*, 31: 507, 1950.
64. Putnam, F. W., Mikaye, A. and Meyer, F. Chemical characterization of abnormal human serum globulins. *Federation Proc.*, 14: 265, 1955.
65. Deutsch, H. F. and Kratschvil, C. H. Relation of so-called pathological proteins to normal serum γ-globulins. *Federation Proc.*, 14: 202, 1955.
66. Griffiths, L. L., Gilchrist, L., King, M. B. and Brews, V. A. L. Cryoglobulin in lymphoid leukosis. *Lancet*, 11: 1286, 1954.
67. Wuhrmann, F. Einige aktuelle klinische Probleme über die Serum-globuline. *Schweiz. med. Wchnschr.*, 82: 937, 1952.
68. Wintrobe, M. M. and Buell, M. V. Hyperproteinemia associated with multiple myeloma. With report of a case in which an extraordinary hyperproteinemia was associated with thrombosis of the retinal veins and symptoms suggesting Raynaud's disease. *Bull. Johns Hopkins Hosp.*, 52: 156, 1933.
69. Osserman, E. F. and Lawlor, D. P. Abnormal serum and urine proteins in thirty-five cases of multiple myeloma, as studied by filter paper electrophoresis. *Am. J. Med.*, 18: 462, 1955.
70. Waldenström, J. Three new cases of purpura hyperglobulinemica. A study in long-lasting benign increase in serum globulin. *Acta med. Scandinav.* (*supp. 266*), 142: 931, 1952.
71. Dörken, H. Primary and secondary purpura hyperglobulinemia. *Acta haemat.*, 10: 340, 1953.
72. Dalgaard, E. B. A case of hyper- and macroglobulinemia accompanied by atypical lymphatic hyperplasia. *Acta path. et microbiol. Scandinav.*, 27: 506, 1950.
73. Bernard, J., Inceman, S., Zara, M. and Christol, D. La dysglobulinémie maligne hémorragipare. *Revue d'hémat.*, 7: 264, 1952.
74. Schubothe, H. Personal communication.
75. Lucey, H. C., Leigh, E., Hoch, H., Marrack, J. R. and Johns, R. G. S. Study of a case of purpura associated with bone changes and formation of a gel in the serum on cooling. *Brit. J. Exper. Path.*, 31: 380, 1950.
76. Schaub, F. Zum Krankheitskild und zur Differentialdiagnose der Macroglobulinämie Waldenström. *Schweiz. med. Wchnschr.*, 82: 890, 1952.
77. Butler, K. R. and Palmer, J. A. Cryoglobulinaemia in polyarteritis nodosa with gangrene of extremities. *Canad. M. A. J.*, 72: 686, 1955.
78. Stein, L. and Wertheimer, E. A new fraction of a cold-susceptible protein in blood of dogs infected with Kala-azar. *Ann. Trop. Med.*, 36: 17, 1942.
79. Pedersen, K. O. Ultracentrifugal Studies on Serum and Serum Fractions, p. 117. Uppsala, 1945. Almqvist & Wiksells.
80. Oncley, J. L., Scatchard, G. and Brown, A. Physical-chemical characteristics of certain of the proteins of the normal human plasma. *J. Physiol. & Colloid Chem.*, 51: 184, 1947.
81. Pillemer, L., Blum, L., Lepow, I. H., Ross, O. A., Todd, E. W. and Wardlaw, A. C. The properdin system and immunity: I. demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena. *Science*, 120: 279, 1954.
82. Rundles, R. W., Cooper, G. R. and Willett, R. W. Multiple myeloma. IV. Abnormal serum components and Bence Jones protein. *J. Clin. Investigation*, 30: 1125, 1951.
83. Bichel, J., Bing, J. and Harboe, N. Another case of hyperglobulinemia and affection of the central nervous system. *Acta med. Scandinav.*, 138: 1, 1950.
84. Wilde, H. and Hitzelberger, A. L. Macroglobulinemia. Clinical features and differential diagnosis. *Blood*, 9: 875, 1954.
85. Atlas, D. H., Cardon, L. and Bunata, J. A note on the use of the Kagan falling drop proteinometer. *Am. J. Clin. Path.* (Tech. Supl.), 7: 21, 1943.
86. Bing, J. Futher investigations on hyperglobulinemia. (I. Occurrence and degree of hyperglobulinemia in the various diseases. The ratio between hyperglobulinemia, hyperproteinemia and hypoalbuminemia. The formolgel reaction). *Acta med. Scandinav.*, 103: 547, 1940.
87. von Bonsdorff, B., Groth, H. and Packalén, T. On the presence of a high-molecular crystallizable protein in blood serum in myeloma. *Folia haemat.*, 59: 184, 1938.
88. Griffiths, L. L., Gilchrist, L., King, M. B. and Brews, V. A. L. Cryoglobulin in lymphoid leukosis. *Lancet*, 2: 1286, 1954.
89. Lüscher, E. and Labhart, A. Blutgerinnungsstörung durch βγ-globuline; zur Kenntnis der Gerinnungsstörungen durch körpereigene Antikoagulanten. *Schweiz. med. Wchnschr.*, 79: 598, 1949.
90. Stefanini, M. Basic mechanisms of hemostasis. *Bull. New York Acad. Med.*, 30: 239, 1954.
91. Morgan, W. S. The probable systemic nature of Mikulicz's disease and its relation to Sjögren's syndrome. *New England J. Med.*, 251: 5, 1954.
92. Harvier, P., Turiaf, J. and Delbarre, F. Purpura hémorragique, syndrome de Raynaud et cryoprotéinémie. *Presse méd.*, 61: 1549, 1953.
93. Grümer, H. D., Grunze, E. and Hörner, H. U-V Spektrophotometrie von Makroglobulinn. *Klin. Wchnschr.*, 33: 588, 1955.
94. Stefanini, M. and Dameshek, W. The Hemorrhagic Disorders. A Clinical and Therapeutic Approach, chap. 10. New York, 1955, Grune & Stratton.
95. Hill, R. M., Dunlop, S. G. and Mulligan, R. M. A cryoglobulin present in high concentration in the plasma of a case of multiple myeloma. *J. Lab. & Clin. Med.*, 34: 1057, 1949.
96. Liljestrand, A. and Olhagen, B. Persistently high erythrocyte sedimentation rate. *Acta med. Scandinav.*, 151: 425, 1955.
97. Kanzow, U., Scholtan, W. and Müting, A. Serologische Differenzierung von Makroglobulinämien. *Klin. Wchnschr.*, 33: 1043, 1955.
98. Long, L. A. Riopelle, J. L., Francoeur, M., Pare, A., Poirier, P., Beorgesco, M. and Colpron, [illegible] Macroglobulinaemia. Effect of macroglobulins on prothrombin conversion accelerators. *Canad. M. A. J.*, 73: 726, 1955.
99. Smith, E. L., Brown, D. M., McFadden, M. L., Buettner-Janusch, V. and Jager, B. V. Physical, chemical, and immunological studies on globulins from multiple myeloma. *J. Biol. Chem.*, 216: 601, 1955.
100. Putnam, F. W. Abnormal human serum globulins. *Science*, 122: 275, 1955.

PHYSICO-CHEMICAL CHARACTERISTICS AND ASSOCIATED CLINICAL DATA OF PUBLISHED CASES OF PROVED, MARKED CRYOGLOBULINEMIA

(Accessory Table)

Part I—Multiple Myeloma

Author and Reference*	Diagnosis	Cryoglobulin Concentration (gm.%)	Temperature of Precipitation (°C.)	Crystal Formation	Gel Formation	Electrophoresis	Ultracentrifuge S constant	Cold Sensitivity	Raynaud's Phenomenon	Cold Purpura or Urticaria	Retinal Vascular Changes	Bleeding Tendency	Anemia	Remarks
Wintrobe and Buell, 1933[1]	.....	7.25	37	+	..	.....	.....	+	+	..	+	+	..	Retinal vein thrombosis
von Bonsdorff, Groth and Packalén, 1938[2]	.....	.....	.....	+	..	.....	For crystalline cryoglobulin, 7.8; some higher molecular weight components present	..	..	..	..	+	+	Two-phase separation—lower viscid layer = ¼–⅕ of whole serum; history of syphilis
Bing, 1940[3]	.....	.....	.....	+	..	.....	Low molecular weight protein (?Bence-Jones) in upper layer serum	..	..	..	..	..	..	Two-phase separation 0°C.; lower syrupy layer had protein content of 17.18 gm.%, and contained some albumin
Flemberg and Lehmann, 1944, 1948[4,5] (Case 1)	.....	1.3 at 0°C.	.....	+	+	.....	Macroglobulins absent	+	..	+	..	..	+	Cold purpura, necrotic skin lesions; vasculitis
Hansen and Faber, 1947[4]	.....	2.25	37.5 [illegible]	..	..	.....	.....	+	+	+	+	+	+	"Aleukemic plasma cell leukemia"
Flemberg, 1948[5] (Case 3)	.....	.....	31	..	..	Whole serum: very large discrete γ globulin peak; increased β globulin also	.....	+	..	+	..	..	+	Cryoglobulin an amorphous precipitate; vesicles and edema of legs when exposed to cold; areas of gangrene of skin; splenomegaly
Hill, Dunlop and Mulligan, 1949[6]	.....	.....	32	..	+	.....	.....	+	..	..	..	..	..	Amino acid composition similar to normal β and γ globulins
Barr, Reader and Wheeler, 1950[7] (Case 2)	.....	5.4–9.8	.....	..	..	γ globulin mobility; homogeneous	γ globulin; (molecular weight 190,000)	+	+	+	+	+	+	.....
Olhagen, 1950[8] (Case 1)	.....	1.4	Room temperature after 48 hr.	+	..	Mobility of γ globulin	7.2 Molecular weight 180,000	..	..	..	..	..	..	Cutaneous discolorations on face, arm, legs; skin biopsy: protein precipitates in capillaries
(Case 2)	.....	0.8	Room temperature after 48 hr.	+	..	Homogeneous, sharp peak; mobility slightly faster than γ globulin	7.1	..	..	..	..	..	..	.....
(Case 3)	.....	0.6	"Immediate on cooling"	+	..	Mobility slightly faster than γ	.....	..	..	..	..	..	..	.....
Rörvik, 1950[9]	.....	.....	33	+	+	.....	.....	+	..	+	..	+	..	γ globulin 4.2 gm.%; cryoglobulin probably a γ globulin

Blades, 1951[10]	.....	.....	22	+	+	.....	.....	..	..	..	..	..	..	Cryoglobulin presumably in γ fraction which was 7.0 gm.%; myeloma suspected because of cryoglobulinemia
Bernard, Inceman, Zara and Christol, 1952[11]	.....	.....	.....	..	+	Mobility of a β globulin	.....	..	..	..	+	..	..	.....
Cugudda, 1952[12]	.....	1.0–1.5	.....	..	+	Tall, sharp peak in α_2 region of supernate, presumably the cryoglobulin	.....	+	..	..	..	..	..	Peripheral gangrene; diffuse arterial and venous thromboses, including visceral thromboses; Sia test, positive result
Brauman, Gregoire, Lambert, Kleyntiens and Danis, 1953[13]	.....	5.3	27	..	+	γ globulin	7.8	..	..	..	..	+	..	.....
Putnam and Udin, 1953[14]	.....	.....	.....	..	..	Mobility of γ globulin	6.6 (molecular weight 167,000)	..	..	..	..	..	..	Related immunologically to gamma globulin
Marshall and Malone, 1954[15]	.....	.....	25	+	+	Mobility of γ globulin	.....	..	..	..	+	..	+	Two-phase separation on cooling; lower layer a gel; purpuric retinal and cerebral changes presumably due to cryoglobulin precipitation
Osserman and Hines, 1954[16]	.....	.....	10	..	+	Mobility slightly faster than γ globulin	5.7	..	..	..	..	+	+	Temporary regression of cryoglobulinemia on treatment with 6-mercaptopurine
Hardy and Putnam, 1955[17]	.....	.....	.....	..	..	Mobility of γ globulin	95% = 6.6 5% = 9.6	..	..	..	..	..	+	Renal damage, uremia; cryoglobulin resembled normal gamma globulin chemically except for absence of terminal amino end-groups such as glutamic acid
Nelson and Neill, 1955[19]	.....	1.4	Room temperature	..	+	Mobility of γ globulin	.....	+	+	..	..	..	..	.....

Part II—Miscellaneous Diseases

Holmberg and Gronwall, 1942[18]	Rheumatoid arthritis	1.3	37	+	..	Slightly faster than γ globulin	6.4–6.8, homogeneous	..	..	..	..	..	..	Non-specific positive reaction to Wassermann test
Atlas, Cardon and Bunata, 1943[19]	"Liver disease"	.....	15–18	..	..	.....	.....	..	..	..	..	..	..	Two-phase separation; lower syrupy layer had total protein 13.0 gm.% as compared with supernate protein 4.97 gm.%
Wertheimer and Stein, 1944[20]	Kala azar: Case 1 Case 2	 1.56 0.3												 Flocculent precipitate Flocculent precipitate
	Bacterial endocarditis	2.5	.....	..	..	.....	.....	..	..	..	..	..	..	.....
	Miscellaneous	.....	.....	..	..	.....	.....	..	..	..	..	..	..	Smaller amounts of cryoglobulin in cases of malaria, chronic nephritis, 3 other cases of kala azar, other cases of bacterial endocarditis
Waldenström, 1944[21]	Macroglobulinemia	.....	6	..	+	.....	19.2 (molecular weight >1,000,000)	..	..	..	+	+	+	Small lymphocytoid cells in marrow; low fibrinogen concentration
Shapiro and Wertheimer, 1946[22]	Periarteritis nodosa	0.27	.....	+	..	.....	.....	..	..	+	..	..	..	Acrocyanosis

PHYSICO-CHEMICAL CHARACTERISTICS AND ASSOCIATED CLINICAL DATA OF PUBLISHED CASES OF PROVED, MARKED CRYOGLOBULINEMIA

(Part II—Miscellaneous Diseases—Continued)

Author and Reference*	Diagnosis	Cryoglobulin Concentration (gm.%)	Temperature of Precipitation (°C.)	Crystal Formation	Gel Formation	Electrophoresis	Ultracentrifuge S constant	Cold Sensitivity	Raynaud's Phenomenon	Cold Purpura or Urticaria	Retinal Vascular Changes	Bleeding Tendency	Anemia	Remarks
Lerner and Greenberg, 1946[24] Lerner and Watson, 1947[23]	Chronic glomerulonephritis	0.8	.....	+	..	Mobility $+3.38\ cm^2\ volt^{-1}sec^{-1} \times 10^{-5}$ acetate buffer pH 4.7, $\mu = 0.1$	.....	+	..	+	..	+	+	.....
Most and Lavietes, 1947[25]	Kala azar	1.9 at 5°C.	.....	..	..	Mobility of γ globulin	.....	..	..	..	..	..	..	Small amounts of cryoglobulins observed in approximately 15 other cases of kala azar (= 50% of cases studied)
Flemberg, 1948[5]..... (Case 2)	Chronic nephritis	.....	Room temperature	+	..	Mobility of γ globulin—sharp peak; homogeneous	.....	+	+	+	..	..	+	Cryoglobulin precipitation only when serum exposed to air (presumably loss of CO_2 with resultant change in pH); false positive reaction to Wassermann test; lymphadenopathy, splenomegaly; no increase in plasma cells of bone marrow
Abrams, Cohen and Meyer, 1949[26]	Lymphosarcoma	36% of total protein	.....	..	..	Mobility intermediate between β and γ globulin	Two components: (*a*) S 15.5 = 85%; (*b*) S 20 = 15%	..	..	..	..	..	..	Serum cryoglobulin identical with that obtained from lymphosarcoma tissue
Bianchi, Giampalmo and Marmont, 1949[27]	"Aleukemic plasmacytosis"	.....	21.5–23.0	..	+	Mobility of γ globulin	.....	..	..	..	+	+	+	Severe pancytopenia; increased serum viscosity
Schwartz and Jager, 1949[28]	Chronic lymphatic leukemia	1.75+ (variable)	10	..	..	Mobility not stated	.....	+	+	+	..	..	+	White amorphous precipitate; cryoglobulin immunologically related to γ globulin
Barr, Reader and Wheeler, 1950[7] (Case 1)	Idiopathic cryoglobulinemia	0.02	.....	..	..	.....	.....	+	..	+	..	..	+	Flocculent precipitate
Dalgaard, 1950[29]	Atypical lymphatic leukemia	.....	22–26	+	..	Mobility between β and γ globulins; homogeneous	.....	..	..	..	..	+	+	Two-phase separation on cooling; lower syrupy layer had protein concentration 13.0 gm.%; serum viscosity increased
Lucey, Leigh, Hoch, Marrack and Johns, 1950[30]	Macroglobulinemia ("reticulosis")	.....	25	..	+	Mobility of β globulin with narrow spiked peak	17.2 (molecular weight 400,000–1,000,000)	..	..	..	..	+	+	Cryoglobulin had β globulin mobility, but did not have lipid content of β globulin; immunologically related to γ globulins
Craig, Waterhouse and Young, 1952[31]	Chronic lymphatic leukemia	0.7	22–28	..	+	Mobility slower than γ globulin (?cryoglobulin-γ globulin complex)	.....	..	..	..	..	+	..	Several episodes of bronchopneumonia; autoimmune hemolytic disease, low-titer cold hemagglutins
Gard, Heller and Malmros, 1952[32]	Macroglobulinemia	.....	.....	..	+	.....	19 (molecular weight 1,000,000)	..	..	..	..	..	..	Sia test +; electron microscopy: spherical particles
McFarlane, Dovey, Slack and Papastamatis, 1952[33]	Macroglobulinemia (plasmacytosis of marrow)	.....	.....	..	+	Homogeneous spike migrating between β and γ globulins	Multiple components (3)—85% S18, 7–8% each of S7 S28	..	..	..	..	+	+	Diffuse plasma cell infiltration marrow. Trace Bence Jones protein in urine. Immunologically no cross reaction with γ globulin

Muirhead, Montgomery and Gordon, 1952[34]	"Hypersensitivity state"	.....	.....	..	..	Prominent β and γ globulin peaks; albumin decreased	.....	..	..	..	..	..	..	Pulmonary vascular sclerosis due to *in vivo* occlusion by precipitated cryoglobulin; syphilis associated; plasmacytosis of bone marrow
Schaub, 1952[35] (Case 1).....	Reticulosarcoma macroglobulinemia	.....	8	..	..	Mobility between β and γ globulins	15.4	..	..	..	..	+	+	Autopsy diagnosis: reticulosarcoma with transition to myeloma; Sia test, negative results; serum showed specific cross reaction with antimacroglobulin rabbit serum
(Case 4).....	Chronic aleukemic lymphadenosis	.....	10	..	+	Mobility of γ globulin	Macroglobulins absent	..	+	..	..	+	+	Serum extremely viscous; 33% lymphoid reticulum cells
Griffiths and Gilchrist, 1953, 1954[36,37]	Lymphatic leukemia	6.15	.....	..	+	Migration between β and γ globulins (distinct from fibrinogen); homogeneous by two-dimensional paper electrophoresis	.....	..	..	..	..	..	+	.....
Harvier, Turiaf and Delbarre, 1953[38]	Idiopathic cryoglobulinemia	2.6	29.5	..	+	Mobility of slow γ globulin; ?non-homogeneous	Macroglobulins suggested by rough solubility screening test	+	+	..	..	+	+	Amino acid analyses showed methionine content to be very low; Bence-Jones protein present in urine; plasmacytosis of bone marrow
Hutchinson and Howell, 1953[39]	Idiopathic cryoglobulinemia	1.6	.....	..	+	.....	.....	..	+	..	..	..	..	Gangrene necessitating amputations; possible myelomatosis
Steinhardt and Fisher, 1953[40]	Hypersensitivity state; idiopathic cryoglobulinemia	4.3	.....	..	+	.....	.....	+	+	..	..	+	..	Recurrent arthritis; lymph node enlargement; replacement of marrow by small lymphocytes
Braunsteiner, Falkner, Neumayer and Pakesch, 1954[41] Petermann and Braunsteiner, 1954[42]	Macroglobulinemia ("reticulosis")	2.5 at 15°C.	.....	..	..	Mobility of γ globulin	Multiple components S>27, S27, S19, S12, S6; whole serum: main components S19, S27	+	..	+	..	+	+	Treated for syphilis; cirrhosis of liver found at autopsy; bone marrow: increased lymphocytes
Feiereis and Schnert, 1954[43]	Paraproteinosis	.....	4	..	+	Mobility of γ globulin	Abnormal 7S globulin sedimenting as two components	..	..	..	..	..	..	Pancytopenia; plasma cell increase in marrow (12%); viscous serum; Sia test, positive reaction
Rundles, Coonrad and Arends, 1954[44]	"Subleukemic lymphatic leukemia"	.....	2	..	+	Mobility intermediate between β and γ globulins	.....	+	+	+	..	..	..	Reduction in cryoglobulin concentration following ACTH therapy
Butler and Palmer, 1955[45]	Polyarteritis nodosa	.....	24	..	..	Mobility of γ globulin	.....	..	..	..	..	..	+	Flocculent precipitate; gangrene of hands and feet due to vascular obstruction by an eosinophilic coagulum; "cryoglobulin inclusion cell" found
Mandema, Van der Schaaf and Huisman, 1955[46]	Macroglobulinemia	4.46	28	..	..	Mobility of γ globulin; homogeneous	19.2 and 30 for isolated cryoprotein	..	..	..	..	+	+	Two-phase separation on cooling with formation of lower syrupy layer; splenomegaly; small lymphocytoid and atypical plasma cells in bone marrow
Volpé and Ogryzlo, 1955[47]	Cryoglobulinemia	1.6	21	..	..	.....	.....	..	..	..	..	..	..	Intracellular inclusions in neutrophils ("cryoglobulin inclusion cell"); concentration of cryoglobulin not altered by treatment with ACTH

* Each author listing is for one case report, unless otherwise noted. References follow this table.

References for Accessory Table

1. Wintrobe, M. M. and Buell, M. V. Hyperproteinemia associated with multiple myeloma. With report of a case in which an extraordinary hyperproteinemia was associated with thrombosis of the retinal veins and symptoms suggesting Raynaud's disease. *Bull. Johns Hopkins Hosp.*, 52: 156, 1933.
2. von Bonsdorff, B., Groth, H. and Packalén, T. On the presence of a high-molecular crystallizable protein in blood serum in myeloma. *Folia haemat.*, 59: 184, 1938.
3. Bing, J. Further investigations on hyperglobulinemia. (I. Occurrence and degree of hyperglobulinemia in the various diseases. The ratio between hyperglobulinemia, hyperproteinemia and hypoalbuminemia. The formolgel reaction). *Acta med. Scandinav.*, 103: 547, 1940.
4. Hansen, P. F. and Faber, M. Raynaud's syndrome originating from reversible precipitation of protein. *Acta med. Scandinav.*, 129: 81, 1947.
5. Flemberg, T. Niagra fall med. patologiskt förändrad blodäggvita. *Nord. med.*, 37: 330, 1948.
6. Hill, R. M., Dunlop, S. G. and Mulligan, R. M. A cryoglobulin present in high concentration in the plasma of a case of multiple myeloma. *J. Lab. & Clin. Med.*, 34: 1057, 1949.
7. Barr, D. P., Reader, G. G. and Wheeler, C. H. Cryoglobulinemia. I. Report of two cases with discussion of clinical manifestations, incidence and significance. *Ann. Int. Med.*, 32: 6, 1950.
8. Olhagen, B. Spontaneous precipitation of crystalline globulin in myeloma serums. *Acta med. Scandinav.* (Suppl. 239), 138: 86, 1950.
9. Rörvik, K. Cryoglobulinemia: a survey and a case report. *Acta med. Scandinav.*, 137: 390, 1950.
10. Blades, A. N. Cryoglobulinaemia in multiple myelomatosis. *Brit. M. J.*, 1: 169, 1951.
11. Bernard, J., Inceman, S., Zara, M. and Christol, D. La dysglobulinémie maligne hémorragipare. *Revue d'hémat.*, 7: 264, 1952.
12. Cugudda, E. Plasmocitoma con crioglobulinemia e trombosi arteriose e venose multiple. *Minerva med.*, 2: 205, 1952.
13. Brauman, J., Gregoire, F., Lambert, P. P., Kleyntjens, F. and Danis, P. Un cas de myelome à cryoglobuline. Etude critique, chimique et physiologique. *Acta clin. Belg.*, 8: 333, 1953.
14. Putnam, F. W. and Udin, B. Proteins in multiple myeloma. I. Physicochemical study of serum proteins. *J. Biol. Chem.*, 202: 727, 1953.
15. Marshall, R. J. and Malone, R. G. S. Cryoglobulinaemia with cerebral purpura. *Brit. M. J.*, 2: 279, 1954.
16. Osserman, E. F. and Hines, M. S. Multiple myeloma with cryoglobulinemia treated with 6-mercaptopurine. *Proc. Am. A. Cancer Research*, 1: 36, 1954.
17. Hardy, S. and Putnam, F. W. Proteins in multiple myeloma. IV. Interaction with metabolic nitrogen. *J. Biol. Chem.*, 212: 371, 1955.
18. Holmberg, C. G. and Gronwall, A. Ein neues krystalinisches Serumglobulin. *Ztschr. f. physiol. Chem.*, 273: 199, 1942.
19. Atlas, D. H., Cardon, L. and Bunata, J. A note on the use of the Kagan falling drop proteinometer. *Am. J. Clin. Path.*, (Tech. Suppl.), 7: 21, 1943.
20. Wertheimer, E. and Stein, L. The cold susceptible globulin fraction of pathological sera. *J. Lab. & Clin. Med.*, 29: 1082, 1944.
21. Waldenström, J. Incipient myelomatosis or "essential" hyperglobulinemia with fibrinogenopenia—a new syndrome? *Acta med. Scandinav.*, 117: 216, 1944.
22. Shapiro, B. and Wertheimer, E. Spontaneous crystallization of a protein from pathological human serum. *Brit. J. Exper. Path.*, 27: 225, 1946.
23. Lerner, A. B. and Watson, C. J. Studies of cryoglobulins. I. Unusual purpura associated with the presence of a high concentration of cryoglobulin (cold precipitable serum globulin). *Am. J. M. Sc.*, 214: 410, 1947.
24. Lerner, A. B. and Greenberg, G. R. A homomolecular serum protein with anomalous solubilities. *J. Biol. Chem.*, 162, 429, 1946.
25. Most, H. and Lavietes, P. H. Kala azar in American military personnel. *Medicine*, 26: 221, 1947.
26. Abrams, A., Cohen, P. P. and Meyer, O. O. The physical properties of a cryoglobulin obtained from lymph nodes and serum of a case of lymphosarcoma. *J. Biol. Chem.*, 181: 237, 1949.
27. Bianchi, V., Giampalmo, A. and Marmont, A. Contributo alla conoscenza della gelificazione plasmatica "a frigore" e della plasmocitosi aleucemica con emogelificazione (gel-plasmocitomatosi). *Minerva med.*, 401: 101, 1949.
28. Schwartz, T. B. and Jager, B. V. Cryoglobulinemia and Raynaud's syndrome in a case of chronic lymphatic leukemia. *Cancer*, 2: 319, 1949.
29. Dalgaard, E. B. A case of hyper- and macroglobulinemia accompanied by atypical lymphatic hyperplasia. *Acta path. et microbiol. Scandinav.*, 27: 506, 1950.
30. Lucey, H. C., Leigh, E., Hoch, H., Marrack, J. R. and Johns, R. G. S. Study of a case of purpura associated with bone changes and formation of a gel in the serum on cooling. *Brit. J. Exper. Path.*, 31: 380, 1950.
31. Craig, A. B., Waterhouse, C. and Young, L. E. Autoimmune hemolytic disease and cryoglobulinemia associated with chronic lymphatic leukemia. *Am. J. Med.*, 13: 793, 1952.
32. Gard, S., Heller, L. and Malmros, H. Electron microscopy of euglobulin from a case of hyperglobulinemia. *Proc. Soc. Exper. Biol. & Med.*, 79: 328, 1952.
33. McFarlane, A. S., Dovey, A., Slack, H. G. B. and Papastamatis, S. C. An unusual case of hyperglobulinaemia. *J. Path. & Bact.*, 64: 335, 1952.
34. Muirhead, E. E., Montgomery, P. O. and Gordon, C. E. Thromboembolic pulmonary vascular sclerosis: report of a case following pregnancy and of a case associated with cryoglobulinemia. *Arch. Int. Med.*, 89: 41, 1952.
35. Schaub, F. Zum Krankheitsbild und zur Differentialdiagnose der macroglobulinämie Waldenström. *Schweiz. med. Wchnschr.*, 82: 890, 1952.
36. Griffiths, L. L. and Gilchrist, L. Cryoglobulinemia in alcoholic cirrhosis. *Lancet*, 1: 882, 1953.

37. Griffiths, L. L., Gilchrist, L., King, M. B. and Brews, V. A. L. Cryoglobulin in lymphoid leukosis. *Lancet*, 2: 1286, 1954.
38. Harvier, P., Turiaf, J. and Delbarre, F. Purpura hémorragique, syndrome de Raynaud et cryoprotéinémie. *Presse méd.*, 61: 1549, 1953.
39. Hutchinson, J. H. and Howell, R. A. Cryoglobulinemia: report of a case associated with gangrene of the digits. *Ann. Int. Med.*, 39: 350, 1953.
40. Steinhardt, M. J. and Fisher, G. S. Cold urticaria and purpura as allergic aspects of cryoglobulinemia. *J. Allergy*, 24: 335, 1953.
41. Braunsteiner, H., Falkner, R., Neumayer, A. and Pakesch, F. Macromolekulare Kryoglobulinämie. *Klin. Wchnschr.*, 32: 722, 1954.
42. Petermann, M. L. and Braunsteiner, H. A cryoglobulin of high sedimentation rate (macroglobulin) from human serum. *Arch. Biochem.*, 53: 491, 1954.
43. Feiereis, H. and Schnert, H-E. Seltene Form einer Paraproteinose. *Klin. Wchnschr.*, 32: 998, 1954.
44. Rundles, R. W., Coonrad, E. V. and Arends, T. Serum proteins in leukemia. *Am. J. Med.*, 16: 842, 1954.
45. Butler, K. R. and Palmer, J. A. Cryoglobulinaemia in polyarteritis nodosa with gangrene of extremities. *Canad. M. A. J.*, 72: 686, 1955.
46. Mandema, E., Van der Schaaf, P. C. and Huisman, T. H. J. Investigations on the amino acid composition of a macroglobulin and a cryoglobulin. *J. Lab. & Clin. Med.*, 45: 261, 1955.
47. Volpé, R. and Ogryzlo, M. A. The cryoglobulin inclusion cell. *Blood*, 10: 493, 1955.
48. Flemberg, T. and Lehmann, J. (Discussion of paper by Waldenström, J.) *Nord. med.*, 23: 1565, 1944.
49. Nelson, M. G. and Neill, D. W. Diffuse myelomatosis with massive cryoglobulinemia. *Irish J. M. Sc.*, 354: 271, 1955.

Monocytic Leukemia*

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SINCE 1913, when Reschad and Schilling-Torgau[1] described the first case of monocytic leukemia, considerable controversy has raged on the subject. For years many authors denied the existence of the disease, claiming that it was a variant of one of the other types of leukemia. However, the clinical and cytologic studies of a host of workers including Dameshek,[2] Clough,[3] Doan and Wiseman[4] and Forkner[5] established its separate identity on a firm basis. By 1942 Evans,[6] in a poll of American hematologists, found overwhelming acceptance of monocytic leukemia as a distinct type. He collected 179 acceptable cases from the literature, and many more have been reported since that time.

Acute monocytic leukemia has been well described. In 1932 Clough[3] was able to present a review of twenty-two cases. He noted that in many of these there was a history of onset with oral infection, gingivitis or angina; the lymph nodes were usually not appreciably enlarged, and the liver and spleen were only moderately enlarged. Two years later these observations were further emphasized by Forkner[5] in a study of his own series of cases. Forkner indicated that ulceronecrotic lesions of the oral cavity were distinctive of monocytic leukemia, and described the lymphadenopathy and enlargement of the abdominal organs as being intermediate between that observed in myelocytic and lymphocytic leukemia. Evans agreed with Clough and Forkner that there are certain distinctive, although not pathognomonic, clinical manifestations of the acute disease, and these features have by now found general acceptance.

Although infrequently mentioned in reviews on this disease, ulcerative lesions of the intestinal tract seem to occur with high frequency. In 1935 Whitby and Christie[7] stated that ulcerations of the alimentary tract were a distinctive feature. Scattered case reports have appeared noting such lesions.[5,8–12] White[13] and more recently Lynch[14] have called particular attention to the ulceronecrotic lesions of the perirectal area. Bonnin[15] believed that the incidence of rectal lesions is common enough to warrant repeated rectal examination of patients in these cases.

Though acute monocytic leukemia has been frequently observed and its clinical features well described, the chronic form of the disease has remained enigmatic. A review of the literature reveals a relative paucity of well documented cases. Many of the reported chronic cases seem in reality to be acute cases with survival a few months longer than usual. The textbook descriptions of the chronic form of the disease are brief. Sturgis[16] states that anemia is usually present, oral lesions are common, and purpura develops in 80 per cent of the cases. Wintrobe[17] describes the clinical course as erratic and notes the difficulty of precise diagnosis. Evans[6] gives a somewhat longer description, mentioning the mildness of initial symptoms, the high incidence of cutaneous lesions, and the occurrence of death due to exhaustion or infection, often in the absence of typical manifestations of leukemia. He states that in patients with leukopenia diagnosis is particularly difficult and is frequently delayed until biopsy or autopsy.

During the past few years our attention has been drawn to a series of patients who have presented an obscure anemia and in whom, after a period of time, unequivocal evidence of monocytic leukemia has developed. We have rather consistently observed certain clinical and laboratory manifestations which seem peculiar to these cases. These observations prompted a review of our cases of monocytic leukemia over the last ten years and the observations form the basis of this report.

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METHODS

In the ten-year period beginning July 1, 1944 twenty-five cases of unequivocal monocytic leukemia have been seen at the Johns Hopkins Hospital. In all of these cases slides of the blood and bone marrow were available for re-examination. Instances in which doubt existed as to the cell type are not included in the series. In every instance it was agreed that the predominant cells in the blood, bone marrow, or both, were clearly recognizable as cells of the monocytic series. In some cases smears of aspirated marrow showed blast cells almost entirely. In these cases diagnosis was established by the presence of large numbers of mature and immature monocytes in the blood. Although some have claimed special characteristics for "monoblasts" as apart from other blast cells, we are not convinced that they can be identified with certainty.

The following criteria were employed for identification of monocytes in smears stained with Wright's stain: (1) large, irregular cells, frequently with pseudopodia; (2) bluish-gray or slate-gray cytoplasm containing tiny lilac granules; (3) irregular, lobulated and folded nuclei, and (4) fine, lacy, lightly stained nuclear chromatin. In addition, the ability to phagocytize India ink particles in fresh preparations was required.

The differentiation of cases of leukemia as acute or chronic is necessarily arbitrary. Our criteria for classifying cases as chronic are as follows: (1) either the total duration of illness was greater than one year, or (2) the bone marrow did not show evidence of acute leukemia at the time of initial examination. The total duration of disease was calculated from the onset of the first obvious symptoms to the time of death. The distinction in time limit between acute and chronic is longer than most others have used. We have used the longer time period (one year) because with modern methods of supportive therapy it is not unusual for patients with acute leukemia, monocytic and other types, to survive for more than six months. However, we have classified as chronic those cases of less than one year's duration in which there were never any hematologic changes of acute leukemia in blood or marrow and in which death appeared to occur prematurely as a result of a secondary complication such as infection.

ACUTE OCCURRENCES

Of the twenty-five cases of monocytic leukemia, seventeen were acute. These cases will be briefly summarized. Ages ranged from sixteen to eighty-three with a mean of fifty-three. Most patients were between forty and sixty-five years. No sex or race was significantly preponderant.

Our experiences with acute monocytic leukemia confirm in all essentials the observations of others as to the distinctive clinical features of this type. Lesions of the oral cavity occurred in thirteen of the seventeen patients. These lesions consisted of hyperplasia, ulceration and hemorrhage of the gums and sometimes of other structures of the oral cavity. Five of our patients developed perirectal lesions. The most common lesion was an anal fissure. In two patients the anal fissures were asymptomatic and would have been overlooked were it not for routine examination. In one of these the fissure disappeared after remission of the disease was accomplished with 6-mercaptopurine but reappeared five months later when the patient's condition relapsed. Perirectal abscess has been a serious complication in two patients. Skin lesions have occurred in only two patients. In one patient these were multiple erythematous, papular lesions, which developed bullous centers and became pustular. In the other case the skin lesion was a hyperplastic infiltration of the beard area. Lymphadenopathy was generally of slight degree except for enlargement of the cervical nodes when there was oral infection. Enlargement of the liver often occurred before detectable enlargement of the spleen. In the two most fulminating cases enlargement of the liver or spleen was never detected clinically. In almost all the patients hepatosplenomegaly was only of moderate degree, and it was unusual for either organ to extend as far as the umbilicus. In the seven autopsied cases liver weight varied from 1,600 to 2,340 gm., and the spleen weighed between 200 and 650 gm.

Most patients had a moderate leukocytosis when first seen but several patients had normal white cell counts. Invariably, unless depressed by therapy, the white cell count rose terminally to levels greater than 100,000. The three patients whose initial white cell count was greater than 100,000 died within three weeks after first being seen and five weeks after onset of symptoms. In most cases small numbers of myelocytes were noted in the blood from time to time. However, we have been unable to correlate the presence of myelocytes with clinical features or prognosis. Nucleated red cells in large numbers were frequently found in the blood smear. Bone marrow examinations have uniformly shown a densely cellular marrow consisting mostly of blast cells, promonocytes and a few mature monocytes. Megakaryocytes were very reduced in number, as were the normal myeloid and erythroid cells.

These cases had a very acute, rapid course.

TABLE 1

Case No. and Reference	Age and Sex	Clinical Features	Laboratory Results	Comment
1, Orr (1933)[8]	40, M	Undiagnosed anemia and leukopenia for 9 mo.; multiple furunculosis; slight lymphadenopathy and hepatomegaly. Duration: 12 mo.	Anemia and neutropenia; 9 mo. after onset developed monocytosis	At autopsy, monocytic infiltration of viscera with atypical multinucleate giant cells
2, Doan, Wiseman (1933)[4]	66, M	Hemorrhage after dental extractions; few small lymph nodes; moderate hepatomegaly; good general health. Duration: 18 mo.	Found to have slight anemia WBC 20,000 to 30,000 with 50 to 60% monocytes 6 mo. after onset	Died of cerebrovascular accident; autopsy showed pleomorphic infiltration of marrow, spleen and lymph nodes with monocytes, eosinophils and plasma cells
3, Marchal, et al. (1934)[22]	73, M	Anemia; slight lymphadenopathy; moderate hepatomegaly. Duration: 22 mo.	Slight anemia; WBC 10,000 to 35,000 with 52 to 83% monocytes	Autopsy showed monocytic, pleomorphic infiltration of marrow and viscera
4, Whitby, Christie (1935)[7]	44, F	Persistent anemia; three exacerbations of fever, oral ulceration and monocytosis; two spontaneous remissions; moderate hepatomegaly. Duration: 12 mo.	During exacerbation WBC 20,000 to 300,000 with 60 to 80% monocytes	Autopsy showed monocytic pleomorphic infiltrations containing megakaryocytes in marrow, viscera and intestinal tract
5, Osgood (1937)[23]	64, M	Persistent fatigue and weight loss; no lymphadenopathy; moderate splenomegaly. Duration: 36 mo.	Anemia; WBC 27,000 with 44% monocytes 18 mo. after onset	Bone marrow smears showed 33% mature and immature monocytes
6, Osgood (1937)[23]	62, M	Multiple furunculosis; epistaxes; no lymphadenopathy; hepatosplenomegaly. Duration: 24 mo.	Marked anemia; 24 mo. after onset WBC 50,000 with 60% monocytes	Autopsy showed diffuse monocytic infiltrations
7, Jacobsen (1942)[24]	60, M	Anemia; splenomegaly; splenectomy done; later developed slight lymphadenopathy and hepatomegaly; bronchopneumonia; pustular exanthem. Duration: 24 mo.	Anemia and neutropenia; postsplenectomy leukocytosis with normal differential; later WBC 100,000 with 55% immature monocytes	Spleen showed monocytic reticulum cells and plasma cells in pulp and follicles
8, Rappoport, Kugel (1947)[25]	28, M	Recurrent neutropenia with hyperplasia of gums, oral ulceration, perianal inflammation, and slight lymphadenopathy and hepatosplenomegaly; splenectomy done after the fourth exacerbation. Duration: 10 mo.	Persistent anemia; myelocytes present at time of neutropenia; WBC 200,000 with 90% monocytes and monoblasts during terminal period	Initial marrow aspiration showed 30% monocytes suggestive of leukemia; later aspirations normal. Spleen showed hyperplasia of reticulum cells with multinucleated giant cells

TABLE 1 (*Continued*)

Case No. and Reference	Age and Sex	Clinical Features	Laboratory Results	Comment
9, Rohkrämer (1950)[22]	17, F	Tonsillitis, hypertrophy of gums, slight lymphadenopathy and hepatosplenomegaly. Duration: 12 mo.	Anemia; neutropenia with only 5 to 15% monocytes; neutropenia made more severe by urethane	Marrow aspiration showed 50% atypical reticulum cells; autopsy revealed monocytic cell infiltration of marrow and viscera
10, Beattie, Seal, Crowther (1951)[27]	55, M	Anemia for 3 yrs.; then erythematous papular skin lesions, gingivitis, moderate lymphadenopathy and hepatosplenomegaly. Duration: 42 mo.	WBC 4,000 to 5,000 with 48% monocytes; terminal blastic blood with thrombocytopenia	Initial marrow showed 15% monocytes; autopsy showed visceral monocytic infiltrations
11, Beattie, Seal, Crowther (1951)[27]	58, F	Erythematous papular skin lesions, slight lymphadenopathy. Duration: 15 mo.	Anemia; WBC 28,000 with 43% monocytes	Marrow showed monocytic infiltration; skin nodule showed monocytoid cell infiltration. No autopsy was performed
12, DiGuglielmo et al. (1953)[28]	55, M	Fever and weakness; granulations on oral mucous membranes, slight lymphadenopathy and splenomegaly. Duration: 12 mo.	WBC 4,000 to 5,000 with 50–90% monocytes throughout course	Marrow showed 57% monocytes; skin biopsy showed monocytic cell infiltration
13, Meacham, Weisberger (1954)[29]	63, F	Symptoms of anemia; splenectomy done after 1 yr.; hepatomegaly and hemorrhagic phenomena. Duration: 43 mo.	Pancytopenia for 30 mo.; terminal monocytosis	Initial marrow aspiration normal; spleen slightly enlarged but normal histologically
14, Meacham, Weisberger (1954)[29]	56, M	Symptoms of anemia; erythematous indurated skin lesions; no lymphadenopathy; moderate hepatosplenomegaly. Duration: 30 mo.	Anemia and neutropenia; terminal monocytosis	Initial marrow showed erythroid hyperplasia; spleen enlarged but histologically normal; liver biopsy revealed no abnormalities

One patient, who did not receive specific antileukemia therapy, lived six months, but usually death ensued within two months. Specific antileukemia therapy in four patients seemed to prolong life a few months. Partial remission of the disease occurred in one of two patients treated with tri-ethylene-melamine, two of five patients treated with cortisone and one of two patients treated with 6-mercaptopurine. In no instance did a complete hematologic remission occur, and the longest survival observed was only eight and half months.

CHRONIC OCCURRENCES

In a review of the literature we have been able to find a total of only fourteen cases which have been presented in detail and which fulfill our criteria for classification as chronic monocytic leukemia. Brief summaries of these cases are tabulated in Table 1. To these we add eight cases of our own.

It will be noted that we have not included several cases that have been considered by others to be chronic monocytic leukemia. Some of these appeared to be cases of acute leukemia. Many cases of "chronic monocytic leukemia" were reported by Marchal et al.,[18,19] Weissenbach[20] and Le Blaye and Chardac.[21] We have not included these because of the incomplete description and the uncertainty of diagnosis. They were characterized by marked leukocytosis, slight anemia, moderate lymphadenop-

TABLE II
CASE 16

Hematology*	May 9, 1950	Sept. 21, 1950	Feb. 16, 1951	Sept. 5, 1951	Jan. 4, 1952	April 14, 1952
Hematocrit	35	32	32	30	26	15
Platelets	136	136	500	108	416	142
WBC	3.5	3.4	3.8	1.3	48	313
Blast cells	0	0	1	3	49	89
Myelocytes	1	0	4	1	10	1
Juveniles	2	9	6	5	4	4
Neutrophils	30	14	16	19	8	3
Basophils	8	0	1	4	0	0
Eosinophils	0	0	0	0	0	0
Lymphocytes	31	29	37	41	15	3
Monocytes	28	42	29	21	2	0
Promonocytes	0	6	6	6	12	0

* In this and subsequent tables platelets and WBC are expressed in 1000 per cu. mm., the remaining items in per cent.

athy, massive splenomegaly, marked radiosensitivity and prolonged survival. While some of these cases were probably instances of monocytic leukemia, it seems likely that others were lymphocytic.

CASE REPORTS

CASE 15. A fifty-eight year old white man (No. 334764) was first seen in October 1944 complaining of one and a half years of easy fatigability. Six months earlier he had been found to be anemic and was troubled by bleeding gums. The anemia had required six transfusions. Persistent low grade fever had developed. Physical examination showed signs of mild weight loss; several teeth were infected and there was moderate pallor. The superficial lymph nodes were not enlarged. The liver edge could be palpated at the costal margin; the spleen could not be felt. The hematocrit value was 35 per cent; the red cells were normocytic and normochromic. The white cell count was 4,150 with 26 per cent neutrophils, 45 per cent lymphocytes and 25 per cent monocytes. The sternal marrow was hypercellular; it was typical of leukemia with massive infiltration by young monocytes and blast cells. In March 1945 the symptoms and signs were unchanged. Blood examination gave the same findings. No further information on the patient was available.

Comments. The bone marrow in this case showed almost complete leukemia replacement by immature monocytes. For at least a year the only abnormality of the blood was mild anemia and monocytosis. The duration of survival is unknown.

CASE 16. A thirty-six year old Negro man (No. 537177), was first seen in May 1950 complaining of three months of malaise, headaches and weakness. Physical examination failed to disclose any significant abnormality. There was neither lymphadenopathy nor hepatosplenomegaly. Blood counts showed mild anemia, leukopenia and monocytosis. (Table II.) The sternal marrow was cellular; the myelocytic and erythrocytic cells were normal but 15 per cent of the cells appeared immature with bluish, vacuolated cytoplasm containing some azure granules and lobulated nuclei and had the appearance of young monocytes. For the next twenty months the patient was chronically ill. The monocytosis and leukopenia persisted. The anemia became progressively more severe and required more and more frequent transfusions. In October 1950 aspirated marrow resembled that previously examined. Extensive studies were performed in an effort to establish some chronic inflammatory disease as the cause of the anemia and monocytosis. All these studies gave negative results. A chronic fistula-in-ano developed. Repeated episodes of folliculitis and furunculosis occurred and persistent induration and draining sinuses developed in the left axilla and inguinal areas. These abscesses and draining sinuses could be improved only temporarily by antibiotics or surgical excision. In September 1951 the patient developed homologous serum jaundice with transient hepatomegaly.

In January 1952 slight enlargement of the liver and spleen was detected. Leukocytosis with many blast cells in the blood now appeared. The aspirated sternal marrow was cellular with many megakaryocytes, no erythroid cells, and 76 per cent blast cells and young monocytes. The white cell count dropped spontaneously to 3,800 but rose again. Nitrogen mustards were given without beneficial effect. The liver and spleen increased in size. The patient died in April 1952 after an illness of over two years.

Comments. For nearly two years the patient had had anemia, leukopenia, monocytosis and multiple soft tissue abscesses. During this time repeated marrow aspirations showed only slight, non-specific abnormalities. Biopsy specimens of the subcutaneous lesions showed many cells, interpreted as macrophages,

which were thought to indicate chronic inflammation. Then, rather abruptly, the patient developed obvious neoplastic invasion of the blood, marrow and other tissues. The final course was that of acute leukemia.

Case 17. A fifty-six year old white man (No. 558767) was first seen in December 1950 complaining of episodes of fever in the afternoon of six months' duration. Anemia had been noted three months prior to admission. Physical examination disclosed pallor of the mucous membranes. No significant lymphadenopathy was noted. The liver and spleen were slightly enlarged. The hematocrit value was 28 per cent; the red cells were normochromic and normocytic. Platelets were 458,000. The white cell count was 2,900 with 26 per cent neutrophils, 59 per cent lymphocytes and 13 per cent monocytes. Sternal marrow smears were cellular. Megakaryocytes were hyperabundant; mature myeloid cells were reduced. There were 10 per cent blast cells and 24 per cent myelocytes; 17 per cent of the cells were large with bizarre irregular nuclei and light blue cytoplasm which contained medium-size reddish granules. Erythrocytic cells were numerous with many binucleate and macrocytic forms.

For the next year and a half the patient was followed elsewhere.* Transfusions were given as necessary. Leukopenia persisted and the liver and spleen became progressively larger. A trial of cortisone had no effect. In October 1953 the fever increased and the patient continued to lose weight. The white cell count was 19,000 with 7 per cent neutrophils, 34 per cent lymphocytes, 57 per cent monocytes and 2 per cent blast cells. The bone marrow showed almost complete replacement by blast cells and monocytes. The patient died in November 1953 after an illness of three and a half years.

At autopsy monocytic infiltration of the bone marrow, lymph nodes, intestinal mucosa, kidneys, pancreas, adrenals and liver was noted. The spleen was diffusely infiltrated, with disappearance of the germinal follicles.

Comments. For two years the patient had had unexplained anemia and neutropenia. The bone marrow six months after onset of disease and three years antemortem showed only general immaturity of cells and a number of unusual cells which resembled monocytes except for unusually prominent cytoplasmic granules. Serious suspicion of leukemia did not arise until the final year. Finally and suddenly, massive generalized neoplastic invasion occurred and the course of the disease was acute and fulminating.

Case 18. A fifty-five year old white man (No. 562129), a nickel plating worker, was first seen in January 1951 complaining of five months of weakness, pallor and weight loss. He had been hospitalized elsewhere and had been found to have had anemia; the bone marrow had been found normal by aspiration. In December 1950 sore throat and leukoplakic patches in the pharynx had developed. Slight lymphadenopathy and hepatomegaly were present. The white cell count was said to be 26,000 with many blast cells and myelocytes. One week of urethane therapy was given, and the patient was referred to the Johns Hopkins Hospital. Physical examination disclosed a large posterior perforation of the nasal septum, an ulcer on the upper gum and follicular exudates on the tonsils. Pea-size superficial lymph nodes were noted. The liver was not felt, spleen was slightly enlarged and a soft mass was felt just inside the anal sphincter. The hematocrit value was 16 per cent; the red cells were normocytic and normochromic. Platelets were 48,000. The white cell count was 6,000 with 1 per cent blast cells, 4 per cent myelocytes, 53 per cent neutrophils, 14 per cent lymphocytes and 27 per cent monocytes. Nucleated red cells, some of which resembled megaloblasts, were seen in the blood smear. Sternal marrow smears were cellular. There were 10 per cent blast cells, 5 per cent mature monocytes and 11 per cent promonocytes; there were 8 per cent erythrocytic cells, most of which were large, orthochromic forms with abnormal chromatin resembling megaloblasts. Many reticulum cells and mitotic figures were present.

Because of the megaloblasts in the marrow, the patient was given large doses of vitamin B_{12}, without effect. Tri-ethylene-melamine, 30 mg., was given over a two week period, and severe leukopenia developed. A second marrow aspiration showed an increase in the number of blast cells, monocytes and reticulum cells. The white count fell to 200. Severe pneumonitis and multiple visceral abscesses, resistant to antibiotics, developed. The patient died elsewhere in March 1951, after an illness of about seven months.

Comments. This case was complicated by overtreatment with TEM, which caused agranulocytosis and probably hastened death. The leukemic invasion of the marrow was only moderate. Of great interest was the persistence of megaloblasts in the marrow.

Case 19. A forty-five year old white woman (No. 567865) was first seen in April 1951 complaining of anemia of eighteen months' duration. Investigations elsewhere had failed to reveal the cause of the anemia, and transfusions had been necessary. Physical examination disclosed slight enlargement of the spleen. No lymphadenopathy or hepatomegaly was noted. The hematocrit value was 29 per cent; the red cells were normochromic and normocytic. Platelets were 438,000. The white cell count was 4,050 with 1 per cent blast cells, 3 per cent myelocytes, 25 per cent juveniles, 32 per cent neutrophils, 13 per cent lymphocytes and 25 per cent monocytes. The sternal marrow was cellular. There were 24 per cent blast cells, 5 per cent mature monocytes and 11 per cent promonocytes. The percentage of undifferentiated myelocytes was increased. Few erythrocytic cells were

* Records were supplied by Dr. J. Heyward Gibbes, Dr. C. L. Cardwell and the Veterans Administration Hospital, Columbia, South Carolina.

TABLE III
CASE 20

Hematology	Jan. 15, 1951	Dec. 4, 1951	Aug. 26, 1952	Dec. 23, 1952	Feb. 18, 1953	Aug. 22, 1953
Hematocrit	25	22	27	29	27	32
Platelets	291	97	132	....	....	49
WBC	7.1	3.8	5.9	4.0	14.7	109
Blasts	0	2	0	0	0	16
Myelocytes	0	2	0	0	0	7
Juveniles	0	1	3	2	2	1
Neutrophils	16	19	12	5	6	0
Basophils	0	0	0	0	0	0
Eosinophils	1	2	0	1	0	0
Lymphocytes	82	70	84	74	55	13
Monocytes	1	4	1	18	37	63
Promonocytes	0	0	0	0	0	0

present, and some of these had abnormal nuclear chromatin resembling megaloblasts.

The patient was followed elsewhere,* feeling moderately well with periodic transfusions. In January 1953, however, hemorrhagic phenomena developed. One month later several bean-size subcutaneous nodules and massive hepatosplenomegaly were noted. No lymphadenopathy was present. The white cell count was 210,000 with almost all blast cells on differential count. The patient died in February 1953 after an illness of over three years. Autopsy was reported to have shown leukemic infiltration of liver, spleen and lymph nodes.

Comments. For three years the course of this patient included refractory anemia with monocytosis, slight symptoms and few physical signs despite the presence of leukemic infiltration of the marrow for at least eighteen months. Then, suddenly, there was massive infiltration of the blood and other tissues with an acute course during the terminal period.

CASE 20. A forty-two year old white man (No. 571013) was first seen in May 1951 complaining of anemia for five months. A sternal marrow biopsy obtained elsewhere was interpreted as showing marrow hypoplasia. Blood transfusions had been given. Physical examination disclosed no lymphadenopathy or enlargement of the liver or spleen. Marked anemia and neutropenia were noted. (Table III.) ACTH gave no beneficial results. In October 1951, 3 per cent blast cells were seen in the blood, and they were seen in small numbers from time to time thereafter. About four blood transfusions per month were necessary. In August 1952 a splenectomy was performed. Examination of the spleen showed only lymphoid hyperplasia. Postoperatively the patient required transfusions, as before, until February 1953 when the transfusion requirement stopped. At the same time leukocytosis and monocytosis gradually developed.

In July 1953 the patient rapidly became acutely ill with sore throat, tender lymph nodes, malaise and fever. Physical examination disclosed several discrete, firm, tender, erythematous raised areas with central bullae over the legs. The right inguinal area was diffusely indurated with draining sinuses. The gums were hypertrophied and ulcerated. The tonsils were very large. The liver was huge. The white cell count was over 100,000 and there were many blast cells. The sternal marrow was densely cellular with massive infiltration of blast cells and reticulum cells. Scanty erythrocytic cells, which were large, orthochromic and had immature nuclei were noted. Nitrogen mustard therapy was of no avail, and the patient died in August 1953 after an illness of two and a half years. Permission for autopsy was refused.

Comments. For over two years the patient's condition had been diagnosed as aplastic anemia. Splenectomy was not helpful either diagnostically or therapeutically. Small numbers of blast cells sporadically appeared in the blood but leukemia was not suspected until the last months when progressive leukocytosis and monocytosis appeared.

CASE 21. A forty-four year old Negro woman (No. 617383) was first seen in September 1952 complaining of fatigue and lassitude of one year's duration. She had been anemic for three months. Physical examination showed no lymphadenopathy or enlargement of liver or spleen. Marked anemia and neutropenia were noted. (Table IV.) Sternal marrow was cellular, and there were over 50 per cent blast cells and promonocytes. Throughout the rest of her life the patient required frequent transfusions. Leukopenia was persistent but the differential count showed a steadily increasing monocytosis. Episodes of severe furunculosis and pneumonitis occurred which re-

* The record was supplied by Dr. Morse Kochtitsky of Nashville, Tennessee.

TABLE IV
CASE 21

Hematology	Sept. 9, 1952	Oct. 17, 1952	Dec. 22, 1952	Mar. 10, 1953	May 2, 1953	June 10, 1953
Hematocrit	11	27	22	24	30	32
Platelets	....	230	194	28	132	182
WBC	1.0	2.5	2.2	3.5	5.0	6.3
Blasts	0	0	0	12	4	3
Myelocytes	0	4	0	11	20	10
Juveniles	7	3	2	2	6	2
Neutrophils	5	8	0	1	1	0
Basophils	0	0	0	0	0	0
Eosinophils	0	0	1	0	0	0
Lymphocytes	79	66	59	55	34	24
Monocytes	6	17	17	1	22	4
Promonocytes	3	2	21	18	11	54
Plasma cells	0	0	0	0	2	3

TABLE V
CASE 22

Hematology	Nov. 22, 1950	Aug. 3, 1951	Nov. 10, 1953	May 24, 1954	Sept. 8, 1954	Jan. 20, 1955
Hematocrit	7.1	7.9	15.8	18.0	23.0	27.0
Platelets	798	492	1,110	436	....	162
WBC	5.4	4.8	5.3	10.2	16.5	30.1
Blasts	0	0	0	0	1	15
Myelocytes	0	0	0	0	2	2
Juveniles	13	22	23	26	20	15
Neutrophils	54	41	47	32	15	15
Basophils	0	0	4	2	1	3
Eosinophils	1	1	2	4	1	2
Lymphocytes	36	22	21	23	14	15
Monocytes	5	14	3	13	40	18
Promonocytes	0	0	0	0	6	15

sponded slowly to antibiotics. An asymptomatic anal fissure was discovered. The patient had a ruptured tubo-ovarian abscess due to coliform bacilli which necessitated prolonged hospitalization. In June 1953 the sternal marrow again showed a preponderance of blast cells and promonocytes. In July 1953 the liver and spleen became palpable. Fever and debilitation increased and the patient died in August 1953 after an illness of fourteen months.

At autopsy the liver and spleen were slightly enlarged. Leukemic infiltrations were found extensively invading the spleen and bone marrow and scattered in the liver, lymph nodes, stomach, small intestine, pericardium and kidneys. The infiltration consisted mostly of monocytic cells but in the marrow and lymph nodes there were considerable numbers of plasma cells and atypical multinuclear cells.

Comments. The disease in this patient was manifested by anemia and neutropenia for at least a year, during which time the bone marrow showed leukemic change. Monocytosis gradually developed, although the total white cell count never increased. Of interest was the appearance of small numbers of plasma cells in the blood during the last months. No acute phase occurred during this illness; instead the course was one of successive infections and progressive debilitation.

CASE 22. A fifty-two year old Negro man (No. 557236) was first seen in November 1950 complaining of three months of weakness and exertional dyspnea. Physical examination disclosed no lymphadenopathy or enlargement of the spleen or liver. Marked anemia and thrombocytosis were noted. (Table v.) The bone marrow was cellular on two examinations. Megakaryocytic hyperplasia was found. The proportion of

erythrocytic cells was reduced but the marrow was otherwise normal. The patient required periodic blood transfusions for the next four years. Examinations of the bone marrow later showed complete absence of erythrocytic cells. In October 1951 splenectomy was performed without beneficial results. The spleen showed only slight lymphoid hyperplasia. In January 1952 ACTH was given without effect.

Beginning in the spring of 1954 there was a gradual rise in the total white cell count and in the percentage of monocytes in the differential count. In May 1954 the bone marrow showed 15 per cent monocytes in a cellular marrow. In January 1955 marked sternal tenderness was discovered. No lymphadenopathy or enlargement of liver or spleen was found. Blast cells and promonocytes had appeared in the blood. The bone marrow now showed almost complete replacement by blast cells and promonocytes.

Comments. For three and a half years the patient was thought to have aplastic anemia, although the thrombocytosis with bizarre platelet forms was thought to be unusual. Then, during a year of close observation, monocytic invasion of the bone marrow and blood developed. Splenectomy was performed and ACTH given, as in Case 20, in the hope that the "aplastic anemia" would be ameliorated. In neither case were these procedures of any value.

OBSERVATIONS

We have reviewed the literature and have been able to find fourteen well documented cases of chronic monocytic leukemia. To these cases eight of our own are added. Our experience is that this disease is not as infrequent as the figures cited would seem to indicate but it is frequently not recognized because, for long periods during its course, there is apt to be a lack of positive, specific symptoms and signs. The manifestations of chronic monocytic leukemia will therefore be discussed in some detail.

The ages of the patients ranged from seventeen to sixty-six but only three were less than forty. Of the twenty-two patients sixteen were males and six were females, which would seem to indicate an appreciable difference in sex. In our experience there was no racial difference.

The patient almost always consulted a physician initially because of non-specific symptoms of weakness, easy fatigability, malaise, weight loss or low grade fever. Sometimes these symptoms seemed in proportion to the degree of anemia present, and sometimes they did not. Occasionally the patient initially presented furnunculosis or other infections. Many patients gave a history of dental difficulty but the acute complaints referable to the oral cavity encountered in acute monocytic leukemia were rarely seen in the chronic form of the disease. These symptoms usually continued throughout the course of the disease, although remissions occasionally were observed.

The initial physical examination generally was completely unrevealing except for the presence of anemia. During the course of the disease many of the physical findings usually associated with leukemia developed. No consistent time pattern was noted for the development of these abnormalities, and in several cases no findings appeared for years. (Cases 10, 11, 15, 20 and 22.) Skin lesions which appeared only during the last six months of the illness, were of two types. The most common type was a multiple erythematous, papular lesion, usually about 2.0 to 5.0 cm. in diameter, which might develop a central pustule. (Cases 7, 10, 14 and 20.) Occasionally, patients developed multiple pea-size subcutaneous nodules, which on biopsy showed leukemia cells and which might be interpreted as reticulum cell sarcoma. (Cases 11, 12 and 19.) The case of Mitchell[30] presented such nodules several months before leukemia became obvious.

Hypertrophy and ulceration of the gums due to leukemic infiltration sometimes developed but was much less common than in the acute form of the disease and usually appeared only as a late manifestation. In two cases gingival hyperplasia and ulceration developed early and remarkable remissions and exacerbations occurred. (Cases 4 and 8.) Lymphadenopathy was slight if present at all. Enlargement of the liver and spleen almost always eventually developed to a slight or moderate degree but was frequently absent early in the disease. No particular time sequence was noted for this enlargement; one organ might enlarge before the other. In two of our patients rectal lesions developed, one a fistula-in-ano and the other an asymptomatic anal fissure. (Cases 16 and 21.) This finding was not observed by others, although the patient in Case 8 had recurrent perianal inflammation.

These patients showed a marked lack of resistance to bacterial infection. In addition to ulceration and infection of the oral cavity and perirectal areas, infected lesions occurred in other areas. Repeated and protracted episodes of furunculosis occurred frequently. (Cases 1, 6, 16, 20 and 21.) Bronchopneumonia was common and was frequently the terminal epi-

sode. (Cases 7, 18 and 21.) In the interesting case of Fairburn and Burgen[31] furunculosis occurred seven months before there was any evidence of a leukemic or hematologic disorder.

Anemia was present at the time of initial examination in every patient. This anemia was of increasing severity and usually required blood transfusions with increasing frequency. Anemia was usually normocytic and normochromic. However, in some cases anemia was macrocytic. (Cases 13, 20 and 21.) Sturgis[16] described a case that was confused with pernicious anemia. In most cases large numbers of nucleated red cells were seen in the blood. No evidence was seen of overt hemorrhage or hemolytic process to account for the anemia. In some cases there was marked reduction in the number of erythroid cells in the marrow, which seemed to provide an obvious explanation for the anemia. However, more frequently the initial marrow examination showed normal numbers of erythroid cells and the anemia could not be explained on the basis of marrow replacement. In several instances abnormalities were observed in the erythrocytic cells. In cases 17 and 19 there were many large orthochromic erythroblasts, with large nuclei containing abnormally clumped chromatin, and many cells with double nuclei. In Case 18 these changes progressed so that half the erythroblasts were indistinguishable from the megaloblasts of pernicious anemia, except that no basophilic forms appeared. Administration of large amounts of vitamin B_{12} had had no effect on these abnormal cells. The changes and the unexplained anemia seem to indicate that in this disease interference occurs with erythrocyte maturation and release. Collins and Rose[32] made similar observations and conclusions in their cases of monocytoid myeloblastic leukemia. Macrocytes and megaloblasts have been observed in myeloid and stem cell leukemia by other investigators as well as by ourselves.

In most cases leukopenia was observed early and it tended to persist throughout most of the illness. Leukopenia was due chiefly to granulocytopenia. The absolute number of lymphocytes was normal, although they were relatively increased. Probably related to the granulocytopenia was the inability of these patients to resist bacterial infections. Granulocytosis in response to infection did not occur, even at a time when the blood was not leukemic and myeloid cells were plentiful in the marrow. Monocytosis was not always present at the onset; sometimes the monocytes in the blood were normal in number and appearance for years. Frequently there was a slight variable monocytosis with the occasional appearance of a few young monocytic forms, which continued for months or years. (Cases 1, 7, 9, 14–17, 19–22.) In most of these cases leukocytosis and monocytosis typical of acute leukemia was noted during the terminal period. In a few, however, leukopenia persisted until death. In only a few cases was there an appreciable absolute monocytosis over an extended period of time. (Cases 2, 5, and 11.)

Most of the cases had thrombocytopenia of some degree, generally not marked until late in the illness. In a few cases early in the disease there was slight thrombocytosis, and megakaryocytes were plentiful in the marrow smears. (Cases 16 and 17.) In Case 22 thrombocytosis persisted for four years. In almost all the cases the platelets in the blood smear were described as showing many giant and bizarre forms. Despite these quantitative and qualitative changes in the circulating platelets, and contrary to apparent popular impression, purpura or other hemorrhagic phenomena were rarely seen in this disease except in the terminal acute phase.

In reference to the various blood elements, we have already alluded to some of the findings of the bone marrow examination. In every case in which the blood contained immature cells the marrow showed leukemic infiltration. In some cases the marrow was found to show leukemic changes at a time when there was no leukemic change in the blood. (Cases 9, 12, 15, 19 and 21.) Frequently, examination of the bone marrow at the time of onset of the illness revealed no evidence of leukemia. (Cases 8, 13, 14, 16, 17, 20 and 22.) At this time the bone marrow usually showed myeloid or erythroid hyperplasia. In two of our cases there were some atypical cells but these were not definitely leukemic or characteristic of monocytes. They were large cells with bizarre nuclei and, at the time, most observers felt that these were atypical myelocytes. Subsequent study of the blood and marrow of these patients indicated that they were monocytic in origin.

A surprising number of patients with anemia, neutropenia and sometimes thrombocytopenia associated with a cellular bone marrow were observed. Frequently this combination led to a

diagnosis of "hypersplenism." In six of these cases splenectomy was performed. (Cases 7, 8, 13, 14, 20 and 22.) Case 8 was interesting in this regard. This patient had four episodes of severe granulocytopenia. During this time several marrow aspirations were performed; one aspiration showed monocytosis and the others showed myeloid maturation arrest. After the fourth episode of granulocytopenia, splenectomy was performed. The histologic sections of the spleen then showed monocytic infiltration and revealed the diagnosis. Likewise, the diagnosis in Case 7 was revealed first by observation of sections of the spleen. In the four other patients who had undergone splenectomy the sections of of the spleen did not demonstrate any significant abnormality. In no case did splenectomy produce amelioration of the disease. During this period of pancytopenia no evidence of a leukemic or neoplastic process was noted. When leukemia became obvious it almost appeared to be a superimposed disease but we prefer to think of the disorder as one continuous process with varying manifestations.

The difficulties in early diagnosis in so many cases of chronic monocytic leukemia are in contrast to the usual cases of chronic lymphocytic or chronic myelocytic leukemia. In a recent study of atomic bomb survivors myelocytes were found in the blood as a precursor to the development of myelocytic leukemia.[33] These myelocytes were found only as the result of routine examination and, by the time clinical symptoms or other hematologic abnormalities appeared, leukemia was obvious.

Some interest has been aroused recently in atypical cases of leukemia in which refractory anemia or pancytopenia is presented. In 1949 Hamilton-Paterson[34] reported three cases in which refractory anemia preceded the development of leukemia. In one of these cases megaloblasts were present in the marrow, and in another macronormoblasts were described. Black and Meynell[35] and Young et al.[36] reported similar cases, which they felt to be myeloid leukemia. Bloch et al.[37] reported twelve patients who had anemia, neutropenia or thrombocytopenia for months or years, then suddenly developed manifestations of acute leukemia and died shortly thereafter. The authors categorized these cases as stem cell leukemia, which they believed was really myelocytic. They termed the prediagnosis period in these patients the "preleukemic phase" and discussed the significance of this syndrome. Meacham and Weisberger[29] more recently have reported ten patients who presented anemia, neutropenia and/or thrombocytopenia. Four of these subsequently developed myelocytic leukemia, two developed monocytic leukemia and one developed reticulum cell sarcoma. These cases are similar to our cases of monocytic leukemia that presented a preleukemic phase.

The significance of this preleukemic phase is not definitely known. Of the twenty-two cases of chronic monocytic leukemia which we have gathered, nine presented a preleukemic phase in which initial examination of the blood and marrow failed to show evidence of leukemia. Eight others presented instances of aleukemic leukemia, and in several of these the initial marrow examination showed only moderate or patchy leukemic infiltration. Only four patients with chronic monocytic leukemia showed leukemic involvement of blood and marrow when first examined. However, in three of these patients the first reported examinations were made six, eighteen and twenty-four months after onset of the illness. The fourth such patient presented acute manifestations, with two remarkable spontaneous remissions. It would seem therefore that the preleukemic mode of onset is typical of monocytic leukemia.

In most of these cases the cellular changes have been more pronounced in the marrow smears than in the fixed pathologic sections. It has been our experience that it is more difficult to recognize moderate to slight changes in marrow cell type in the fixed sections. Even at death, when the diagnosis of monocytic leukemia seems obvious, the pathologic material has sometimes led to confusion. In two patients subjected to autopsy we have observed prominent pleomorphism of the cellular infiltrates in the marrow, spleen, lymph nodes and other viscera. Varying numbers of plasma cells, myelocytes, eosinophils, atypical multinucleated cells (some resembling Reed-Sternberg cells) and megakaryocytes may be seen. This pleomorphism found at necropsy, at times even resembling Hodgkin's disease, has been well described by Marchal,[22] Dameshek,[38] Orr,[8] Doan and Wiseman,[4] Forkner,[39] Osgood,[23] Mallory[40] and Herbut.[41]

A similar pathologic picture has been found in cases of so-called histiocytic medullary reticulosis described by Böhne,[42] Giffin and Watkins,[43] Scott and Robb-Smith,[44] Asher,[45] Evans,[46]

Civin et al.[47] and Paull and Phillips.[48] These cases in every way resemble monocytic leukemia except for the lack of leukemic cells in the blood. Many have regarded this syndrome as an aleukemic form of monocytic leukemia.[49,50] This viewpoint would seem to be strengthened by the finding of many cases of monocytic leukemia in which invasion of the marrow occurred long before overt invasion of the blood. Two of our patients (Cases 15 and 21) never developed leukocytosis, although they had immature monocytes in the blood. Some authors have interpreted this phenomenon as systemic reticuloendotheliosis ending in monocytic leukemia. A reversal of this process was seen in the remarkable case of Anderson and Roberts[51] in which blood and marrow remission of leukemia was followed by development of solid tumors. In these instances, however, there has been no difficulty in adducing abnormality of the marrow, although an exact diagnosis might not have been thought possible. It is well to remember that precise classification of all neoplasms is not always possible and perhaps not always wise, despite whatever self-satisfaction such categorization may bring. In this regard Custer[52] has written of the apparent relationship and resemblance, clinical and pathologic, amongst the various leukemias and lymphomas. He has well emphasized that these neoplastic processes have a common origin in cells of the reticuloendothelial system, and these cells may retain multipotential qualities throughout life.

In the patients who presented a preleukemic phase the diagnosis was very obscure at the time of initial examination and was revealed only by time. Of the patients observed by us one appeared to have hypoplasia of the marrow. In three patients the marrow was normally cellular; in two of these the only abnormality was the presence of some young cells which were difficult to classify, and in the other the absence of erythroid cells was the only abnormality. These cases were believed to have various forms of aplastic or refractory anemia.

In fact, the preleukemic phase of our cases and of the cases of other investigators appears to be indistinguishable clinically and pathologically from aplastic anemia. In studies of the pathology of many cases diagnosed as aplastic anemia Thompson et al.[53] and Rhoads and Miller[54] found that in most instances the marrow was of normal or increased cellularity and that there were increased percentages of immature cells. Bomford and Rhoads[55] collected over thirty cases of aplastic anemia, of which most were classified idiopathic, and some had known exposure to a possibly toxic agent, in all of which a cellular marrow was found. Clinically, many of these cases were similar to some of the cases in the preleukemic phase which we have presented. Several of their patients later developed leukemia. This association of aplastic anemia and leukemia would seem to be more than chance.

In studies of nineteen cases of severe chronic benzol toxicity with depression of blood elements Mallory et al.[56] found that most patients had a cellular marrow with increased immaturity of the cells. Mallory commented particularly on the general immaturity and anaplasia of the marrow cells and stated that it was of a degree he had never before seen in non-neoplastic states. Two of his patients had developed leukemia; the incidence of leukemia is increased in those exposed to benzol.[57] Experimental benzol intoxication in animals produces depression of the blood elements and varying degrees of marrow hypoplasia and hyperplasia with increases in the percentage of immature primitive cells.[58,59] Martland[60] found at autopsy hyperplastic bone marrow with immature and primitive cells in six cases of radium poisoning. Warren and Dunlap[61] stated that small repeated doses of radiation may induce bone marrow hyperplasia, whereas in humans[62] and animals[63] exposed to a single large amount of radiation there is initial bone marrow hypoplasia and regeneration proceeds from surviving primitive reticuloendothelial cells.

It is dangerous to attempt to draw direct correlations of this sort yet we are impressed with the similarity of the marrow response to some known toxic agents and the preleukemic phase of certain cases of leukemia. Depending on the time-dose relationship, these toxic stimuli may cause hyperplasia with interference in the normal maturation and release of the marrow cells, hyperplasia of the marrow with prominence of more primitive reticuloendothelial cells, or complete hypoplasia of the marrow. In the preleukemic cases neoplastic change eventually takes place and sooner or later becomes clinically manifest. One might reason that the aplastic anemia phase and the leukemic phase are separate and distinct entities and that aplastic anemia merely induces increased susceptibility to the development of

leukemia. This viewpoint may be correct. However, for several reasons we prefer to think of these cases as representing one continuous disease process. They may be due to a neoplasm already present but too occult to be morphologically discernible. It would also seem reasonable to speculate that there is wide variation of host response to the leukemic stimulus or stimuli which is reflected in the varying modes of onset. Almost all of the cases of preleukemic phase which we have presented have been considered unusual instances of aplastic anemia because of various atypical features. In the various cases these unusual features were manifested by periodic release of small numbers of blast cells into the blood, gross abnormalities of platelet and red cell maturation, unusual cells in the marrows, splenomegaly, and the like. In several of our adequately followed cases, although the terminal picture was sudden in onset and fulminating in development, the entire clinical and pathologic course, when viewed in perspective, was one of insidious, progressive, leukemic change. As Kaplan[64] has recently pointed out in experimentally induced animal leukemia, there is no sudden irreversible cellular change but rather a train of events during the course of which an irreversible change may occur.

SUMMARY

1. Seventeen cases of acute monocytic leukemia are briefly summarized. These cases conform to the usual description, being characterized by a high incidence of ulceronecrotic lesions of the oral cavity and perirectal area, slight lymphadenopathy and moderate hepatosplenomegaly.

2. Fourteen cases of chronic monocytic leukemia have been found in the literature, and to these we have added eight cases of our own. These cases did not conform to any specific pattern.

3. The most common type was that which presented an initial preleukemic phase. This is characterized by anemia and other cytopenias together with a cellular, non-leukemic bone marrow. During this phase these cases may resemble aplastic anemia clinically but there are progressive, although slow, changes which finally culminate in obvious leukemia.

4. Many cases of chronic monocytic leukemia follow a leukopenic or aleukemic course. At times there may be little leukemic invasion of the blood despite widespread infiltration of the bone marrow and other organs. These cases closely resemble the medullary reticuloses.

5. The rarest type of chronic monocytic leukemia is that in which monocytosis, of considerable degree and consisting of mature cells, is present in the blood for long periods of time.

6. Some of the questions raised by these variations in course are discussed, in particular the relationship of the preleukemic forms to aplastic anemia. The significance and full meaning of this form of onset is not understood.

ADDENDUM

Since the preparation of the foregoing article another similar case has been observed in a twenty-eight year old man. After having undiagnosed anemia for a year, the appearance of pancytopenia led to bone marrow aspiration and the correct diagnosis. In the three months since diagnosis was established, there has been no detectable enlargement of any of the organs. Leukopenia has persisted, but at times up to 40 per cent of the cells have been immature monocytes. During the past month severe hemolytic anemia with a negative reaction to the Coombs' test has been the most troublesome manifestation. A recent sternal marrow aspiration disclosed a cellular marrow composed almost entirely of half erythroblasts and half immature monocytes. The hemolytic activity is partially suppressed by steroids.

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REFERENCES

1. Reschad, H. and Schilling-Torgau, V. Über eine neue Leukämie durch echte Übergangsformen (Splenozytenleukämie) und ihre Bedeutung für die Selbständigkeit dieser Zellen. *München. med. Wchnschr.*, 60: 1981, 1913.
2. Dameshek, W. Acute monocytic leukemia: review of the literature and case reports. *Arch. Int. Med.*, 46: 718, 1930.
3. Clough, P. W. Monocytic leukemia. *Bull. Johns Hopkins Hosp.*, 51: 148, 1932.
4. Doan, C. A. and Wiseman, B. K. The monocyte, monocytosis, and monocytic leukosis. *Ann. Int. Med.*, 8: 383, 1934.
5. Forkner, C. E. Clinical and pathological differentiation of the acute leukemias with special reference to acute monocytic leukemia. *Arch. Int. Med.*, 53: 1, 1934.

6. Evans, T. S. Monocytic leukemia. *Medicine*, 21: 421, 1942.
7. Whitby, L. and Christie, J. Monocytic leukemia. *Lancet*, 1: 80, 1935.
8. Orr, J. W. Monocytic leukemia: two cases. *Lancet*, 1: 403, 1933.
9. Wainwright, C. W. and Duff, G. L. Monocytic leukemia. *Bull. Johns Hopkins Hosp.*, 58: 267, 1936.
10. Campbell, A., Henderson, J. and Croom, J. Monocytic leukemia with myeloid hyperplasia and localized tumor formation. *J. Path. & Bact.*, 42: 617, 1936.
11. Klumpp, T. and Evans, T. Monocytic leukemia. Report of eight cases. *Arch. Int. Med.*, 58: 1048, 1936.
12. Miller, B. Monocytic leukemia simulating cancer of the stomach. *M. Bull. Vet. Admin.*, 15: 307, 1939.
13. White, E. Monocytic leukemia: case report. *M. J. Australia*, 2: 167, 1941.
14. Lynch, M. J., Monocytic leukemia. *Canada M. J.*, 70: 670, 1954.
15. Bonnin, J. A. Monocytic leukemia with chloroma-like tumors. *M. J. Australia*, 1: 835, 1951.
16. Sturgis, C. C. Hematology, 1st ed. Springfield, Illinois, 1948. Charles C Thomas.
17. Wintrobe, M. M. Clinical Hematology, 3rd ed. Philadelphia, 1951. Lea & Febiger.
18. Marchal, G., Bargeton and Mahoudeau. Sur huit cas de leucémie chronique à monocytes. *Bull. et mém. Soc. méd. d. hôp. de Paris*, 2: 1467, 1933.
19. Marchal, G., Porge, J. and Dennewald. Sur les leucémie à monocytes. *Sang*, 11: 561, 1937.
20. Weissenbach, R. J. Leucémie chronique à monocytes, avec manifestations cutanées. *Bull. et mém. Soc. méd. d. hôp. de Paris*, 2: 1658, 1933.
21. Le Blaye and Chardac, J. Sur quelques cas de leucémie chronique à mononucléaires. *Bull. et mém. Soc. méd. d. hôp. de Paris*, 2: 1706, 1938.
22. Marchal, G., Lemoine, J. and Bloch-Michel, R. Leucémie chronique à monocytes, sans splénomégalie, ni adénomégalie. *Sang*, 8: 694, 1934.
23. Osgood, E. E. Monocytic leukemia: report of six cases and a review of one hundred and twenty-seven cases. *Arch. Int. Med.*, 59: 931, 1937.
24. Jacobsen, K. M. Monozytenleukose. *Acta med. Scandinav.*, 111: 30, 1942.
25. Rappoport, A. E. and Kugel, V. H. Monocytic leukemia: case report illustrating variations in the clinical picture. *Blood*, 2: 332, 1947.
26. Rohkrämer, H. Zur Klinik und Pathologie der aleukämischen Systemreticulose. *Ztschr. Klin. Med.*, 146: 310, 1950.
27. Beattie, J. W., Seal, R. M. E. and Crowther, K. Chronic monocytic leukemia. *Quart. J. Med.*, 20: 131, 1951.
28. DiGuglielmo, G. Morrelli, A. and Maurea, C. Istioleucemia cronica. *Haematologica*, 37: 1, 1953.
29. Meacham, G. C. and Weisberger, A. S. Early atypical manifestations of leukemia. *Ann. Int. Med.*, 41: 780, 1954.
30. Mitchell, L. A. Malignant monoblastoma; variant of monocytic leukemia. *Ann. Int. Med.*, 8: 1387, 1935.
31. Fairburn, E. A. and Burgen, A. S. V. The skin lesions of monocytic leukemia. *Brit. J. Cancer*, 1: 352, 1947.
32. Collins, D. H. and Rose, W. M. The nature of anemia in leukemia. *J. Path. & Bact.*, 60: 63, 1948.
33. Moloney, W. C. and Lange, R. D. Leukemia in atomic bomb survivors. *Blood*, 9: 663, 1954.
34. Hamilton-Paterson, J. L. Pre leukemic anemia. *Acta haemat.*, 2: 309, 1949.
35. Black, A. B. and Meynell, M. J. Aleukemic myeloid leukemia presenting as aplastic anemia. *Brit. M. J.*, 1: 1430, 1951.
36. Young, L. E., Platzer, R. F. and Lawrence, J. S. Unusual anemia associated with chronic myeloid leukemia. *Acta haemat.*, 5: 345, 1951.
37. Block, M., Jacobson, L. and Bethard, W. Preleukemic acute human leukemia. *J. A. M. A.*, 152: 1018, 1953.
38. Dameshek, W. Aleukemic reticulosis. *Folia Hemat.*, 49: 64, 1933.
39. Forkner, C. E. Monocytic Leukemia in Symposium on Blood and Blood Forming Organs. Madison, 1939. University Wisconsin Press.
40. Mallory, T. Case report #23021. *New England J. Med.*, 216: 65, 1937.
41. Herbut, A. A. and Miller, F. R. Histopathology of monocytic leukemia. *Am. J. Path.*, 23: 93, 1947.
42. Böhne, C. and Huismans, L. Beiträge zur Kenntnis der chronischen leukämischen Reticuloendotheliosen. *Virchows Arch. f. path. Anat.*, 283: 575, 1932.
43. Griffin, H. and Watkins, C. Distinction between splenic anemia and subleukemic splenic reticuloendotheliosis. *Am. J. M. Sci.*, 188: 761, 1934.
44. Scott, R. B. and Robb-Smith, A. H. T. Histiocytic medullary reticulosis. *Lancet*, 2: 194, 1939.
45. Asher, A. Histiocytic medullary reticulosis. *Lancet*, 1: 650, 1946.
46. Evans, T. S., Cipriano, A. P. and Ferrell, E. H. Reticulo-endotheliosis or stem-cell leukemia: case report. *Connecticut State M. J.*, 13: 1128, 1949.
47. Civin, H., Gotshalk, H. C., Okazaki, K. Histiocytic medullary reticulosis. *Arch. Int. Med.*, 94: 375, 1954.
48. Paull, A. M. and Phillips, A. M. Systemic reticuloendotheliosis in the adult male. *Ann. Int. Med.*, 41: 363, 1954.
49. Freud, P., Plachta, A., Speer, F. D. and Luhby, A. L. Leukemic xanthomatosis. *Am. J. Dis. Child.*, 88: 43, 1954.
50. Gray, J. D. and Taylor, S. Acute systemic reticuloendotheliosis terminating as a monocytic leukemia. *Cancer*, 6: 333, 1953.
51. Anderson, C. D. and Roberts, G. B. S. Case of acute leukemia with remission and development of chloromata. *Glasgow M. J.*, 35: 95, 1954.
52. Custer, R. P. Borderlands dim in malignant disease of the blood forming organs. *Radiology*, 61: 764, 1953.
53. Thompson, W. P., Richter, M. N. and Edsall, K. S. An analysis of so-called aplastic anemia. *Am. J. M. Sc.*, 187: 77, 1934.
54. Rhoads, C. P. and Miller, D. K. Histology of the bone marrow in aplastic anemia. *Arch. Path.*, 26: 648, 1938.
55. Bomford, R. R. and Rhoads, C. P. Refractory anemia: clinical and pathological aspects. *Quart. J. Med.*, 10: 175, 1941.
56. Mallory, T. B., Gall, E. A. and Brickley, W. J.

Chronic exposure to benzene: pathological results. *J. Indust. Hyg. & Toxicol.*, 21: 355, 1939.
57. Hueper, W. C. Recent developments in environmental cancer. *Arch. Path.*, 58: 475, 1954.
58. Latta, J. S. and Davies, L. T. Effects on blood and hemopoietic organs of the rat of administration of benzene. *Arch. Path.*, 31: 55, 1941.
59. Steinberg, B. Bone marrow regeneration in experimental benzene intoxication. *Blood*, 4: 550, 1949.
60. Martland, H. S. Occurrence of malignancy in radioactive persons. *Am. J. Cancer*, 15: 2435, 1931.
61. Warren, S. and Dunlap, C. E. Effects of radiation on the blood and the hemopoietic tissues. *Arch. Path.*, 34: 562, 1942.
62. Liebow, A. A., Warren, S. and DeCoursey, B. Pathology of atomic bomb casualties. *Am. J. Path.*, 25: 853, 1949.
63. Schlumberger, H. G. and Vazquez, J. J. Pathology of total body irradiation in the monkey. *Am. J. Path.*, 30: 1013, 1954.
64. Kaplan, H. S. On the etiology and pathogenesis of the leukemias. *Cancer Research*, 14: 535, 1954.

Seminar on Allergy

Diagnostic Methods for Allergic Diseases*

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THE diagnosis of allergic disease is twofold: recognition of the presence of allergic disease and identification of the specific agent or allergen causing the disease in the particular patient. This paper deals with the latter aspect of diagnosis. There are no general diagnostic measures or tests for the differential diagnosis of allergic diseases. Certain features, such as periodicity, familial incidence and eosinophilia of the blood and secretions, are common to enough of the various diseases of allergy to be helpful but none is pathognomonic of allergy, and the differential diagnosis depends primarily on knowledge of the clinical features of each disease entity.

Despite the introduction of many new drugs for the symptomatic relief of allergic disease, satisfactory long-term management usually depends upon determining the causative allergens affecting the individual patient. These agents may be inhaled dusts, such as pollens, mold spores and animal danders, foods, drugs, substances adsorbed through the skin, or infective agents such as bacteria and fungi. A large proportion of allergic patients are affected by several different allergens. In addition to the allergen causing the basic sensitization, attacks of allergy may be precipitated by non-specific irritants such as smoke fumes and damp air. These secondary factors do not act as antigens or give positive skin reactions. Recognizing them helps in the handling of the case but the main treatment must usually be directed to the actual causative antigens. The determination of the causative allergen, or allergens, is made by correlating the clinical history, physical examination for evidence of infections, and skin tests with specific allergens. In some cases further trials of change of diet or environment may be required before a specific diagnosis can be reached.

History of Allergy. A careful history of the time and circumstances of attacks is the first step in specific diagnosis. The relationship of symptoms to season, to particular houses or locations, to occupation or other activities, to diet or drugs, gives valuable clues as to possible causative factors. The history also calls attention to sensitizations of unusual degree, so that due care may be used in testing with such allergens.

The history should also include a detailed account of all potential allergens with which the patient has contact—pets, pillows and other bedding, occupational contacts, diet—so that these will not be overlooked in subsequent tests.

Proper recording and interpretation of the history requires some knowledge of the technical uses of common allergens, for example, commercial doughnuts often contain cottonseed antigen but cottonseed oil does not; mixed fertilizer may contain castor bean; women's hair preparations may contain flaxseed or vegetable gums, and so forth.

If a single allergen produces the disease, a detailed history may clearly establish the cause. If several different factors are involved, deductions based on history alone are apt to be misleading but reconsideration of the history in the light of skin reactions is more revealing.

Tests For Determining Specific Causes. Most of the tests for identification of specific allergens depend upon reproducing the allergic reaction on a limited and controllable scale by suitable intentional exposure of the patient to the suspected agent.

With a few notable exceptions, such as the fixed drug eruptions, allergic sensitization affects many parts of the body to some degree, although the ordinary clinical manifestations may be localized to one organ particularly exposed to the antigen or unusually rich in fixed antibody content. For example, in hay fever the symptoms are ordinarily limited to the nasal mucosa, which is normally most exposed to contact with

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antigen. However, if antigen is injected into the skin, it reacts readily with an urticarial wheal; and if too large a dose is injected, producing a constitutional reaction, edema of the hands and face, asthma and even uterine cramps may show that many other tissues are sensitive although never reacting to ordinary exposure to the allergen.

Since the skin is very commonly involved in allergic sensitization, is readily accessible to experiment and observation, and can react without disturbance of the vital functions, it is the most frequently chosen site for provoking allergic reactions as tests. Various methods may be employed to bring the antigen into contact with the sensitized cells of the skin. In the *scratch test* it is applied to a scratch which breaks the outer cornified layer of cells. In the *puncture test*[1] a drop of antigen solution is placed on the surface of the skin and introduced by pricking the skin with a needle. The *intracutaneous test* is performed by injecting a small amount of antigen into the superficial layers of the skin with a hypodermic needle. These three methods are used for eliciting the wheal and erythema type of skin reaction characteristic of the immediate allergic reactions, such as atopy and anaphylaxis. The intracutaneous test is also suitable for eliciting delayed bacterial reactions such as the tuberculin reaction. The *patch test*, in which allergen is placed on the unbroken skin surface and allowed to remain for twenty-four hours or longer, is applicable only to agents capable of penetrating the skin surface, and to delayed forms of reaction. It is particularly useful in contact dermatitis when this is the natural route of exposure but also serves to elicit the tuberculin reaction.

Various mucosae have also been utilized as sites of allergy tests. *Conjunctival tests*, in which the antigen is dropped into the conjunctival sac, were described by the earliest students of hay fever, and have been employed not only for the diagnosis of respiratory allergy but also as a test of serum sensitivity. *Nasal tests*, performed by introducing dissolved or powdered antigen into one side of the nose, are occasionally used[2,3] and some authors have recommended *bronchial tests* in which the patient inhales an aerosol of antigen.[4]

In atopic sensitization, such as hay fever, asthma and infantile eczema, and in human anaphylactic sensitization, the serum of the patient usually contains skin-sensitizing antibodies, or reagins, capable of producing specific local passive sensitization, the Prausnitz-Küstner reaction,[5] when injected into normal human skin. This method of passive transfer permits demonstration of allergic reactions to specific antigens under controlled conditions in the skin of a volunteer subject, rather than in the patient himself.

Other approaches to diagnosis of specific allergies employ more or less controlled exposures of the body as a whole to the allergen. These have been used in the study of food allergy in various ways. The elimination diet attempts to correlate the occurrence of allergic symptoms with alternate periods of avoidance and eating of certain foods. The food diary method allows a varied diet, of which the patient keeps an accurate record in conjunction with a record of symptoms. Some authors have attempted to refine these clinical observations of the symptoms following ingestion of a specific food by observing associated effects on the leukocyte count (leukopenic index[6]) or the pulse rate.[7] However, the latter two methods have not been proved to be more specific than simple observation of symptoms.

Skin Tests for Immediate Wheal Type Reactions. As previously mentioned, the scratch, puncture and intracutaneous methods are used to elicit this type of reaction, which is characterized by an urticarial wheal surrounded by a zone of erythema. The scratch and puncture methods introduce minute and unmeasurable amounts of antigen, and for this reason a strong solution of antigen must generally be used to produce clear-cut reactions. The intracutaneous test permits injection of a fairly definite volume but the antigen solution must be highly diluted to avoid an excessive reaction. Tests by any of these three methods may occasionally cause severe general allergic reactions in the highly sensitive patient, and those performing the tests must be thoroughly familiar with this danger and the methods of avoiding or minimizing it. There is no doubt that the intracutaneous method involves greater risk of a general reaction than the scratch or puncture tests. A few fatal reactions to scratch tests have been reported and a considerably larger number from intracutaneous tests.[8] Most of these reactions occurred before the dangers of the method were fully understood and resulted from the use of antigen solutions or methods that are not now considered acceptable.

When the tests are performed by a physician

well trained in the field, with proper precautions, severe general reactions are rare. If the intracutaneous method is used it is important to know the dilution of each antigen which is safe for testing the average patient and also to realize that weaker extracts must be used first in patients whose history suggests an unusual degree of sensitization. Intracutaneous tests should always be done on the arm or thigh where a rubber tourniquet may be effectively applied to prevent further adsorption of antigen in case an excessive reaction occurs. They should be performed in groups of not more than six at a time and the reactions to each group observed before the next is injected, the total at one sitting rarely exceeding twenty-four.

The results obtained by the intracutaneous method are far more definite and reproducible than by the scratch method. The former permits introduction of a fairly uniform volume of antigen solution, with relatively little trauma to the skin; the latter introduces a variable and undetermined amount of antigen with greater trauma, so that a control test is necessary. As a result, patients with ordinary degrees of allergy show a considerable range in the size of reactions to intracutaneous tests, while the differences in appearance of various reactions to scratch tests are less marked. The relative inefficacy of the scratch test is illustrated by the fact that it has not been found reliable as a means of detecting allergy to horse serum in patients to be treated with antitoxin.

The intracutaneous method also permits a rough grading of the degree of sensitivity to an allergen by comparing the size of skin reactions to solutions of various strengths. In the scratch or puncture test, on the other hand, a strong extract must usually be employed to produce a definite reaction, and estimation of the degree of sensitivity from the size of the reaction is difficult because of the uncertainty as to the amount of antigen introduced.

Because of these differences in safety and accuracy some workers in the field prefer the intracutaneous test and others the scratch test, while a few employ both methods, doing scratch tests first and intracutaneous tests later. It is the opinion of the author that the physician relatively inexperienced in the field may well employ the safer scratch method but that well trained workers will gain more satisfactory information from the more accurate intracutaneous method, using the scratch tests as a preliminary only in patients believed to be unusually sensitive to a particular antigen, or when using a new antigen with which they are not thoroughly familiar.

In evaluating the intracutaneous test as evidence of clinical sensitization to an allergen there are several points to be considered: (1) the reproducibility of reactions by the same observer and also by others using different antigen solutions and often slightly different technic, (2) non-allergic factors affecting the skin reaction, and (3) correlation of the reaction of the skin to an allergenic extract with the actual occurrence of symptoms when the patient is exposed to the antigen.

Reproducibility of Results. When a patient is tested intracutaneously with an antigen to which he reacts, the size of the reaction in the skin depends upon the amount of antigen injected and the depth at which it is injected. Most workers aim to inject 0.01 to 0.02 ml. of antigen extract as superficially as possible. The volume injected can be measured fairly accurately with a tuberculin syringe but unavoidable error is often introduced by leakage along the path of the needle. The depth of injection is not measurable; visible distention of the skin to form a bleb or wheal 2 to 3 mm. in diameter is generally considered evidence that the depth was not too great. Because of these conditions, reading the reaction by measuring the wheal and erythema implies a precision that often cannot be proved by repetition of the tests. Grading the reactions as slight, moderate, marked and marked-active, or one plus to four plus, is sufficient for all practicable purposes and yields readings that can usually be duplicated on repetition with the same technic and allergen extract.

When the tests are repeated by others both quantitative and qualitative differences in reactions to skin tests are frequently noted. The discrepancy is usually greatest in the slight and moderate (one or two plus) reactions. To some extent this reflects differences in technic and in grading the observed reactions but the most important differences result from the use of different antigen solutions. There are no government or other uniform standards for the preparation, purity and potency of allergen extracts, and no unanimity of opinion prevails as to the proper dilutions for intracutaneous tests. Since most of the antigens used for intracutaneous testing are readily soluble in alkaline solutions, preparation of an extract sufficiently potent for intracutaneous testing is not difficult. However,

the many different methods of expressing potency, in terms of weight of material extracted, total nitrogen, protein nitrogen, and on various arbitrary scales, make comparison of extracts from different sources difficult and leave the choice of a suitable dilution for testing a matter of experience with the particular extract. Crude extracts from many allergenic materials contain impurities which may irritate non-allergic skin and must be eliminated by dialysis and other chemical means if reliable results are to be obtained on testing. Finally, some of the antigens used are unstable even with refrigeration so that frequent replacement is essential. It is only by constant attention to these problems that reliable results may be obtained by skin tests, and until methods are standardized, somewhat different results will be obtained by different workers.

Non-allergic Factors Affecting Skin Reaction. While the intracutaneous test is intended to measure the immunologic phenomenon of sensitization the reaction observed is the result of changes in local blood flow and capillary permeability, probably as a result of histamine release. Theoretically it may be affected by the amount of locally bound histamine available for release and by the reactivity of the blood vessels to histamine.

In practice, it is usually found that infants give relatively small skin reactions[9] so that the reactions obtained on passive transfer (Prausnitz-Küstner reaction) may be considerably larger than those observed directly on the patient. Aged persons with atrophic skin may also give sluggish reactions. Even among healthy young adults there is a considerable variation in reactivity, patients with fair skin and light or red hair tending to show somewhat larger reactions. All of these factors require judgment in evaluation of the results of skin tests, rather than literal "reading" of the diagnosis.

Various drugs may inhibit the skin reactions. Epinephrine and ephedrine greatly reduce the response, as do relatively large doses of antihistaminic drugs. The steroid hormones and corticotropin have little if any effect.

Correlation of Skin Reactions with Clinical Symptoms. Subject to the conditions mentioned, a consistently positive reaction to intracutaneous tests is evidence of allergy of the skin to the allergen extract. The diagnostic value of skin tests depends on the degree of correlation between this skin sensitivity to the prepared extract and the sensitivity of the shock organ on natural exposure to the allergen. In the common atopic diseases this correlation is great enough to make skin tests a valuable tool in allergic diagnosis but not such as to permit a strictly literal interpretation of the skin reactions.

One factor of prime importance is the amount of antigen reaching the shock organ in an active state after normal exposure. In asthma, for example, inhaled allergens may be deposited directly on the sensitized mucosa, while food antigens undergo great chemical changes in cooking, digestion and metabolism before reaching the bronchi through the circulation. A baker who reacts strongly to a skin test with wheat flour may have severe asthma when he inhales a small amount of flour at a bakery but may be able to eat bread freely without symptoms. The skin reaction is "clinically significant" as regards inhalation but not ingestion.

In general, the correlation between reactions to skin tests and the occurrence of clinical symptoms is greatest in the case of inhaled allergens. Even here the relationship is far from perfect, and the skin tests must always be considered in conjunction with the clinical history in arriving at a diagnosis. In most cases a marked (three plus) reaction to an inhaled allergen indicates potential clinical sensitivity, although not necessarily the occurrence of symptoms on every exposure. On the other hand, definite symptoms may be associated with slight or moderate skin reactions. It is generally stated that some patients who are clinically sensitive to a pollen or other inhaled allergen may give no reaction to intracutaneous tests with the allergen.[10,11] This is most often true of infants and young children in the early stages of developing respiratory allergy. The coexistence of clinical respiratory allergy with completely negative skin tests in adults who have had symptoms for several years is sufficiently rare to call for careful consideration of the evidence before arriving at a definite diagnosis. In some cases the allergen extracts used in testing may be found to be at fault, in others the symptoms may actually be due to another allergen encountered at the same time as the one originally suspected. The conjunctival test may be helpful in cases in which the history is not confirmed by skin tests.

By correlation of the results of the skin tests and the history it is usually apparent that certain skin reactions are confirmed as clinically significant by occurrence of symptoms at times of

exposure to the allergen, while others are not. In the case of pollen antigens this is apparent from the season of symptoms. Patients who give a history of hay fever only during the ragweed pollen season often show skin reactions not only to this antigen but also to tree and grass pollens. Occasionally the latter may be as large or even larger than the reaction to the test with ragweed. In such cases only the diagnosis (and treatment) of ragweed hay fever is warranted but the physician should realize that the clinical significance of the tree and grass pollen reactions will depend greatly on the intensity of exposure to these pollens. If the patient moves from the city to the country these pollens, previously considered not clinically significant, may cause definite hay fever. Symptoms may also be caused by the tree and grass pollens in years when they are unusually prevalent because of weather conditions. There is, therefore, no sharp distinction between significant and "false positive" skin reactions to pollens. The difference is between those allergens which are important and those which are not in causing symptoms in the individual patient and the particular exposure.

In cases in which there are skin reactions both to pollens and to non-seasonal allergens, such as house dust, but the symptoms are essentially limited to the pollen season, evaluation of the importance of the skin reactions to the non-seasonal factors is not always easy. Many such patients have a mild allergic rhinitis throughout the year which is apparent on rhinologic examination but causes no great discomfort; the aggravation of symptoms during the pollen season is so marked that they think of the condition as hay fever. In those cases in which the symptoms tend to continue for some weeks after the actual end of the pollen season the patients often do better if both the pollen and the important non-seasonal factors are considered in the treatment.

When a patient reacts definitely to skin tests with several non-seasonal allergens to which he is exposed much of the time, and has persistent symptoms of varying intensity, evaluation of the significance of each is not easy. Elimination of any one factor may not appreciably affect the symptoms. On the other hand, if elimination or treatment of all the factors simultaneously produces a marked degree of relief, it is then far simpler to determine by careful trial if exposure to any one causes a recurrence. For example, a patient with asthma who reacts to skin tests with feathers, dog and house dust may be reluctant to accept the significance of the dog reaction since symptoms persist during periods of separation from the dog. The only practical procedure is to assume that all three reactions are important. If avoidance of exposure to feathers and dogs, with treatment for dust, results in relief of the asthma it is then usually apparent whether renewed contact with dogs will produce symptoms.

Evaluation of reactions to skin tests with foods is far more difficult than in the case of inhaled allergens, as indicated by the previous discussion of baker's asthma. If a patient regularly has symptoms of allergic disease within a few minutes or less than an hour after eating a particular food, skin tests with it will almost invariably show definite reactions. However, a considerable proportion of the foods causing skin reactions in patients with asthma or allergic rhinitis can be eaten by these patients without apparent effect. On the other hand, in occasional cases foods which are shown by repeated trials to produce allergic reactions may give completely negative skin reactions.

Some of the false positive reactions to foods may be due to inactivation of antigens by cooking. Extracts of food allergens are ordinarily prepared from raw materials, yet many foods are customarily eaten only in the cooked form. Various processes of cooking will alter the antigens in different degrees. Some patients sensitive to egg can tolerate a hard boiled egg but not a soft boiled egg. Some idea of the effect of cooking may be obtained by repeating the food tests showing positive reactions with allergen extracts which have been heated in a water bath at 100°c. for thirty to sixty minutes but this makes no allowance for the variety of procedures used in cooking different foods.

Since the false positive skin reactions may also be caused by foods eaten raw, it appears probable that digestion plays an important part in inactivating ingested allergens. Most of the common food allergens lose their antigenicity when digested *in vitro* with proteolytic enzymes.[12] On the other hand, certain food allergens, such as peanuts and eggs, may be shown by a modification of the Prausnitz-Küstner reaction to be absorbed in significant amounts.[13] If a site in the skin of a non-allergic person is passively sensitized with serum of a patient highly allergic to one of these antigens, eating the specific food is followed in about a half hour by a reac-

tion at the sensitized site. While this experiment has been repeatedly confirmed, it requires the serum of an unusually sensitive patient and gives little idea of the amount of antigen which gains access to the peripheral circulation in an active form. If considerable amounts of food allergens were absorbed in an active state, one would expect the skin of patients who react to skin tests to show some response when the same food is eaten.

In regard to the "false negative" skin reactions, it has been suggested by Cooke[14] that the delayed allergic reactions occurring several hours after the eating of foods giving negative results to the skin tests may be due to the formation of antigens of different specificity during the process of digestion of the food proteins. In a few carefully studied cases Cooke has shown that patients with such delayed clinical reactions to food gave immediate skin reactions to proteoses derived from digestion of the suspected food, but no reaction to extracts of the original food itself. Blamoutier reported similar findings.[15] This approach, which is rather cumbersome for routine use because of the many intermediate stages in protein digestion, has not been widely tested and the extent to which the principle is applicable remains uncertain.[12,16] In practice, these relatively rare cases of delayed food allergy have most often been recognized by careful history taking and dietary trials.

A number of immediate allergic reactions, most often to shell fish and berries or other fruits, are also associated with negative skin reactions to intracutaneous tests.[17,18] These discrepancies are usually due to the fact that the antigens of the foods mentioned are extremely unstable so that no method of preparation or preservation of extracts for intracutaneous testing yields a reliable allergen extract. In the case of these foods a scratch test with the actual food, in fresh or quick-frozen form, often shows a clear-cut reaction when intracutaneous tests with available prepared antigen solutions are negative.

Because of the relatively poor correlation between the reactions to skin tests with foods and the occurrence of clinical symptoms from eating them, it is important to confirm the significance of skin reactions to foods by clinical trial. In some cases the clinical history suffices; a suspicion based on previous experience and then confirmed by a definitely positive reaction to the skin test may reasonably be considered significant. If there has been no suspicion in the past, and skin tests show positive reactions to foods eaten occasionally or frequently, it is well to eliminate these foods from the diet until the patient has been free of symptoms for a week or two, then add one suspected food at a time and observe the results. If elimination from the diet of all the suspected foods is not accompanied by any notable decrease in symptoms, they presumably are not the major causes of symptoms although they may still play a secondary part in the total allergic picture.

Dietary Tests for Food Allergy. Because of these inherent errors in food tests some physicians interested in the diseases due to food allergy have largely or completely abandoned the use of skin tests with food allergens. However, the alternative procedures all require weeks of observation, as compared to one or two hours for the performance of a reasonable number of food tests. Consequently practical considerations favor the use of skin tests as a starting point in the diagnostic study, to be followed if necessary by clinical trials.

If skin tests are not done or have not proved helpful, two general methods of dietary trial may be considered for the diagnosis of suspected food allergy; elimination diets and the food diary. If symptoms are present continuously or almost daily, the elimination diet is the preferred procedure. The food diary is more applicable to allergic diseases in which periodic attacks occur at intervals of several days or a week.

Elimination diets are designed to exclude all probable allergenic foods. Variety is necessarily limited and balanced nutrition is of secondary importance since the diets are designed to be used for only a week or two without change. The logical starting point is the "synthetic" diet from which all allergenic foods are excluded by the use of pure sugars, mineral salts, synthetic vitamins, water and a protein hydrolysate such as protolysate® or amigen.®[19] If symptoms are not relieved after four or five days on this diet, they cannot be attributed to food allergy. However, this diet is so unpalatable that many patients prefer to receive it by nasal tube or intravenously, and it is more common to start with a less drastic procedure.

Many different diets have been advised, most of which are modifications of two basic types: (1) a diet composed of only four or five foods which are statistically not common allergens, such as lamb, rice, peas and pears, (2) a more

varied diet, from which the most commonly allergenic foods, such as milk, eggs and wheat (or all cereal products) are excluded.[20] Starting with a diet of either type, if symptoms are relieved in four or five days, they are presumed to be due to one of the foods that have been eliminated, and an attempt is made to identify the offending foods by adding one at a time to the diet at intervals of four days. If the initial diet does not produce relief, a change is made to another diet not including any of the same foods. In order to avoid confusion the use of medicines for symptomatic relief should be kept to a minimum and no other changes of therapy made at the same time the diet is begun.

The food diary method consists simply of having the patient record all of the foods and beverages taken at each meal (and between meals) and the time of occurrence of symptoms. After several attacks have been recorded an attempt is made to correlate them with the foods eaten during the preceding twelve to twenty-four hours. The diet may be completely unrestricted or partially restricted, but the success of the method obviously depends on periodic recurrence of symptoms.

The apparent simplicity of both of these methods is somewhat misleading. Each approach will occasionally produce striking results but in a great number of patients the findings are inconclusive and confusing. Probably this reflects the small number of allergic patients (except for infantile eczema and the immediate reactions to foods which are obvious from the history) in which symptoms are due primarily to foods. When the attention of both physician and patient is focused on foods, the occurrence of symptoms due to inhaled allergens, intercurrent infections or non-specific secondary irritants may lead to serious misinterpretations which can be corrected only by repetition over many weeks of observation. It is essential that a critical attitude be maintained.[21] To be significant, an effect of ingesting or eliminating a food should be observed in three successive trials unless the severity of symptoms produced makes this unwise. Since the observations in both procedures are largely made by the patient, it is difficult to avoid his personal bias in evaluating the results. For purposes of investigation, various procedures have been devised to introduce foods in capsules, strongly flavored mixtures, or through a nasal tube, without the patient's knowledge of the specific food being given.[22,23] These are not practical until other measures have narrowed the suspicion to a very few foods but they may be useful for confirmation.

Evaluation of Infective Factors. It is well known that the symptoms of respiratory allergies may be precipitated by infections of the respiratory system. There is also clinical evidence that certain cases of urticaria[24] and atopic dermatitis[25] are due wholly or in part to infection. Various authorities differ as to whether such attacks are due to specific allergy to infective agents or to a non-specific effect of infection on allergic disease basically due to extrinsic allergens. The preponderance of evidence, a description of which is outside the scope of this paper, indicates that each of these mechanisms applies in some cases.

In determining the importance of the factor of infection in such allergic diseases skin tests are of little value. Intracutaneous tests with bacterial antigens, vaccines or filtrates only rarely show immediate urticarial reactions of the characteristic atopic type. Delayed inflammatory reactions of the tuberculin type are indicative of previous infection which, in the case of the common respiratory organisms, is so prevalent that it has no diagnostic value. The only instance in which skin tests with bacterial antigens may be considered valid evidence of bacterial allergy is when inadvertent injection of too large a dose of antigen causes exacerbation of the symptoms of the disease. Since such reactions are often delayed eight to twenty-four hours after the test, and may persist for several days, deliberate provocation of them is rarely justified. Evaluation of the infective factor, therefore, is primarily on the clinical grounds of history and a careful physical examination, with any necessary x-rays.

Environmental Tests. In cases of respiratory allergy the presence of both skin reactions to inhaled allergens and evidences of infection often leaves doubt as to which type of factor is most important. In this instance the environmental test is helpful. This consists simply of moving the patient from his home environment to a relatively bare and dust-free hospital room, equipped with air filters during the pollen and mold spore seasons. No medications are given regularly but symptomatic measures of relatively brief action, such as epinephrine, are given as necessary for relief. If the allergic disease is due primarily to extrinsic factors, marked improvement should be apparent in three or four days.

Persistance of symptoms suggests the predominance of intrinsic, usually infective factors.

Diagnosis of Drug Allergy. In the diagnosis of allergy to non-protein drugs, skin tests are of relatively little value.[26] Intracutaneous tests give urticarial reactions in some patients with the immediate anaphylactic type of sensitization to penicillin,[27] thiamine and quinine. A definitely positive reaction indicates that the patient is highly sensitive but a negative reaction does not exclude the possibility of such sensitization. Patch tests with drugs are valuable in the diagnosis of contact dermatitis caused by topical medications. In some forms of dermatitis medicamentosa due to systemic medication patch tests may give positive reactions if the drug is sufficiently absorbed through the unbroken skin. Here again, a positive reaction is evidence of allergy but a negative result does not exclude it.

With the exception of these specific types, skin tests are not helpful in the diagnosis of drug allergy. In the great majority of drug sensitizations, skin tests with the causative drug give completely negative results. This is the case in both immediate reactions, such as asthma due to aspirin, and delayed reactions to penicillin and other drugs. In the more violent types of drug reaction, typified by asthma due to aspirin, skin tests entail a great risk of precipitating an attack even though no local reaction is elicited, and are therefore not indicated.

Except for the contact dermatitis type, the diagnosis of drug allergy must be based on knowledge of the manifestations of sensitization produced by each drug. In general a well grounded suspicion is adequate reason to avoid use of the same drug again. When there is a strong indication for further use of the same drug, skin tests rarely aid in the decision; cautious trial of small doses is usually the only method of proving the diagnosis.

Passive Transfer Tests. By the Prausnitz-Küstner method of passive transfer[5] it is possible to perform skin tests for allergy on a normal substitute whose skin has been passively sensitized with serum of the patient. This procedure is advantageous for testing infants and small children, patients whose skin is so widely involved with eczema or other disease as to be unsuitable for direct tests, patients with marked dermatographia, and patients with severe and persistent asthma requiring almost constant use of epinephrine. It may also be used to test with particular antigens to which the patient is believed to be so sensitive that direct tests might be dangerous. There is no risk of a general reaction in the passively sensitized substitute.

Practical points to be considered are: (1) the patient must be free of syphilis, infectious hepatitis and other transmissible diseases; (2) the serum must be kept sterile by suitable technic; and (3) the test substitute must be free of allergic disease. Since passive sensitization is strictly limited to the sites where serum is injected, control tests of the substitute's own reaction are made at the time of testing.

As a rule, essentially the same antigens react on passive transfer tests as in the skin of the patient himself. The same variations in the physiologic reactivity of the skin of different individuals are noted, except when the substitutes are selected on the basis of youth and healthy skin, and good reactors are used on repeated occasions. In the case of infants and persons with unusually sluggish skin the reactions noted by passive transfer may be somewhat larger than those elicited by direct tests on the patients, but few if any additional reactions are obtained by this method. If the patient has normal adult skin some of the reactions obtained by direct tests may fail to be reproduced in passive transfer. Usually these are slight (one plus) reactions of doubtful clinical significance. However, there is no evidence of significantly greater correlation between the occurrence of clinical symptoms and the reactions to passive transfer tests than in the case of direct tests. The results of both must be evaluated with consideration of the history and clinical observations.

Mucous Membrane Tests. Application of allergen extracts or powdered allergens to the conjunctiva has been used as a test of allergy to pollens and other inhaled allergens in the hope that the reaction of this membrane might be better correlated with causation of clinical symptoms than the skin reactions.[28] Since it is desirable to have one eye as a control, the method is limited to the use of one antigen at a time and is usually employed to confirm doubtful skin tests or to investigate further a suspicion based on the history and not confirmed by skin tests. On theoretic grounds it appears possible that sensitivity of the mucosae occasionally may be associated with negative skin reactions but there is little evidence that any significant number of such cases occur. In practice, the reactions obtained by the skin and eye tests are

usually similar, except that the concentration of antigen required to elicit the eye reaction is greater than that for the intracutaneous test and less than that for the scratch test. The probability of detecting additional cases of sensitization by the eye test therefore depends largely on whether skin tests are done by the scratch or intracutaneous method.

Tests on the nasal[2,3,29] and bronchial mucosa[4,29] are more difficult to perform and interpret. Despite their theoretic advantages for the diagnosis of respiratory allergy, they have not been established as practical routine procedures.

SUMMARY

The many different methods utilized in the specific diagnosis of allergic sensitizations reflect the fact that no one approach may be relied upon to furnish satisfactory results in all cases. While skin tests of various types play an important part in such diagnosis, it is important to realize that they are only one aspect of the study, to be evaluated in conjunction with the history and careful examination of the patient for evidence of infective factors. The conclusions reached must furnish a reasonable explanation of the time and circumstances of occurrence of symptoms, and reflect sound judgment as well as technical skill.

REFERENCES

1. Freeman, J. Hay Fever, A Key to the Allergic Diseases, p. 256. London, 1950. William Heineman.
2. Duke, W. W. Allergy, Asthma, Hay Fever, Urticaria and Other Manifestations of Reaction. St. Louis, 1925. The C. V. Mosby Co.
3. Efron, B. G. and Penfound, W. T. A nasal test with dry pollen in the diagnosis of seasonal hay fever. *J. Allergy*, 2: 43, 1930.
4. Schiller, I. W. and Lowell, F. C. The inhalation test as a diagnostic procedure, with special emphasis on the house dust allergen. *J. Allergy*, 23: 234, 1952.
5. Prausnitz, C. and Küstner, H. Studien über die Ueberempfindlichkeit. *Centralbl. f. Bakt.*, 86: 160, 1921.
6. Vaughan, W. T. The leukopenic index as a diagnostic method in the study of food allergy. *J. Lab. & Clin. Med.*, 21: 1278, 1936.
7. Coca, A. F. Familial Non-Reaginic Food Allergy, 3rd ed. Springfield, 1954. Charles C Thomas & Co.
8. Vaughan, W. T. and Black, J. H. Practice of Allergy, 3rd Ed., p. 153. St. Louis, 1954. The C. V. Mosby Co.
9. Carey, P. N. and Gay, L. N. Skin reactions in infants. *J. Allergy*, 5: 488, 1934.
10. Coca, A. F., Walzer, M. and Thommen, M. Asthma and Hay Fever, p. 334. Springfield, 1931. Charles C Thomas & Co.
11. Vaughan, W. T. and Black, J. H. Practice of Allergy, 3rd Ed., p. 175. St. Louis, 1954. The C. V. Mosby Co.
12. Wacker, I. C., Wetmore, A. and Adkinson, J. Sensitization tests with digestive products of protein. *Arch. Int. Med.*, 32: 323, 1923.
13. Walzer, M. Studies on absorption of undigested proteins in human beings; simple direct method of studying absorption of undigested protein. *J. Immunol.*, 14: 143, 1927.
14. Cooke, R. A. Protein derivatives as factors in allergy. *Ann. Int. Med.*, 16: 71, 1942.
15. Blamoutier, P. Allergie digestive, sensibilization a un product de desintegration de la proteine alimentaire. *Presse méd.*, 53: 162, 1945.
16. Bloom, S., Markowe, H. and Redner, B. Studies in food sensitivity. II. The effect of protein digestion on antigenicity of foods as determined by skin tests and clinical food trials. *J. Allergy*, 23: 64, 1953.
17. Tuft, L. and Blumstein, G. Studies in food allergy. *J. Allergy*, 13: 574, 1942; 15: 346, 1944.
18. Ancona, G. R. and Schumacher, I. C. The use of raw foods as testing materials in allergic disorders. *California Med.*, 73: 473, 1950.
19. Olmstead, W. H., Harford, C. G. and Hampton, S. F. Use of synthetic diet for food allergy and typhoid. *Arch. Int. Med.*, 73: 341, 1944.
20. Rowe, A. H. Elimination Diets and the Patient's Allergy, 2nd ed. Philadelphia, 1944. Lea & Febiger.
21. Ingelfinger, F. J., Lowell, F. C. and Franklin, W. Gastro-Intestinal allergy. *New England J. Med.*, 241: 303 and 337, 1949.
22. Loveless, M. H. Milk allergy: a survey of its incidence, experiments with a masked ingestion test. *J. Allergy*, 21: 489, 1950.
23. Loveless, M. H. Allergy to corn and its derivatives. Experiments with a masked ingestion test for its diagnosis. *J. Allergy*, 21: 500, 1950.
24. Fink, A. I. and Gay, L. N. A critical review of 170 cases of urticaria and angioneurotic oedema followed for a period of 2 to 10 years. *Bull. Johns Hopkins Hosp.*, 55: 280, 1934.
25. Kesten, B. M. Allergic eczema. *New York State J. Med.*, 54: 2441, 1954.
26. Sherman, W. B. Drug allergy. *Am. J. Med.*, 3: 586, 1947.
27. Boger, W. P., Sherman, W. B., Schiller, I. W., Siegal, S. and Rose, B. Allergic reactions to penicillin. *J. Allergy*, 24: 383, 1953.
28. Peshkin, M. M. A dry pollen ophthalmic test in pollen asthma and hay fever patients negative to cutaneous tests. *J. Allergy*, 3: 20, 1931.
29. Feinberg, S. M., Stier, R. A. and Grater, W. C. A suggested quantitative evaluation of the degree of sensitivity of patients with ragweed pollinosis. *J. Allergy*, 23: 387, 1952.

Clinico-pathologic Conference

Recurrent Burning Retrosternal Pain

STENOGRAPHIC reports, edited by Amoz I. Chernoff, M.D., and W. Stanley Hartroft, M.D., of weekly clinico-pathologic conferences held in the Barnes and Wohl Hospitals are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

THE patient (No. 258845), a white salesman, fifty-three years of age, was admitted to the medical service of Barnes Hospital on August 20, 1955, complaining of a burning pain in the chest of five hours' duration. For three years prior to admission the patient had had frequent episodes of mild burning retrosternal pain occasionally accompanied with slight dyspnea. These episodes lasted for thirty to sixty minutes and always occurred at night. The pain was never severe enough to prevent sleeping. It never occurred with physical exertion or following meals. On the day prior to admission the patient drove 300 miles in an automobile. His meals during that day consisted entirely of milk. For supper he had a sandwich and a glass of beer, and during the evening he drank three cocktails. At 1:00 A.M., five hours prior to admission, he noted the onset of burning retrosternal pain which was more severe than usual. For the first time the pain radiated to his left shoulder and was accompanied with a feeling of numbness in the left arm, marked dyspnea and orthopnea. These symptoms lasted for two hours and then subsided. An hour later his symptoms reappeared with the same severity as before, and he was brought to the hospital.

Between the ages of nine and seventeen years the patient had had thirteen episodes of inflammatory rheumatism without subsequent deformity or other apparent sequelae. He gave a history of having had a positive serologic test for syphilis in his youth and stated that he had been treated. The details were obscure. For about twenty-five years the patient smoked two to three packages of cigarettes per day. He frequently drank alcoholic beverages socially.

On admission physical examination revealed the temperature to be 36.4°C.; pulse, 75; respirations, 16; blood pressure, 135/85. The patient was a large, plethoric, slightly obese middle aged man lying flat in bed in no distress. There was no cyanosis or sweating. There was no clubbing of the fingers. Examination of the head, neck, eyes, nose and throat was not remarkable. The lungs were clear to auscultation and percussion. The precordium was quiet and the heart did not appear to be enlarged by percussion. The rhythm was regular, the heart sounds were of poor quality, and no murmurs were heard. Examination of the abdomen and genitalia revealed no abnormalities. The peripheral pulses all felt strong, and no edema was noted. The neurologic examination was within normal limits. No rectal examination was made.

The laboratory data were as follows: blood count: hemoglobin, 16.9 gm. per cent; white blood count, 9,950 per cu. mm.; packed cell volume, 49 per cent; differential count: band forms, 5; segmented forms, 56; lymphocytes, 37; monocytes, 2. The red blood cells and platelets appeared normal. Corrected erythrocyte sedimentation rate, 20 mm. per hour (Wintrobe). Urinalysis: specific gravity, 1.016; reaction, 7.5; protein, negative; sugar, negative; 3 to 5 white blood cells and an occasional red blood cell per high power field. Stool examination: brown, formed, guaiac negative. Blood cardiolipin: negative. Blood chemical determinations: nonprotein nitrogen, 26 mg. per cent; fasting blood sugar, 89 mg. per cent. A roentgenogram of the chest was interpreted by the radiologist as showing calcified hilar lymph nodes bilaterally and intercalary calcification of D10 and D11 intervertebral discs. An electrocardiogram revealed inversion of the T waves in leads V_1 through V_4 and was interpreted as showing anterior myocardial ischemia. (Fig. 1.)

The patient's treatment consisted of complete bedrest, coronary precautions and phenobarbital 30 mg. every six hours. On this regimen he was asymptomatic for eight days. During this

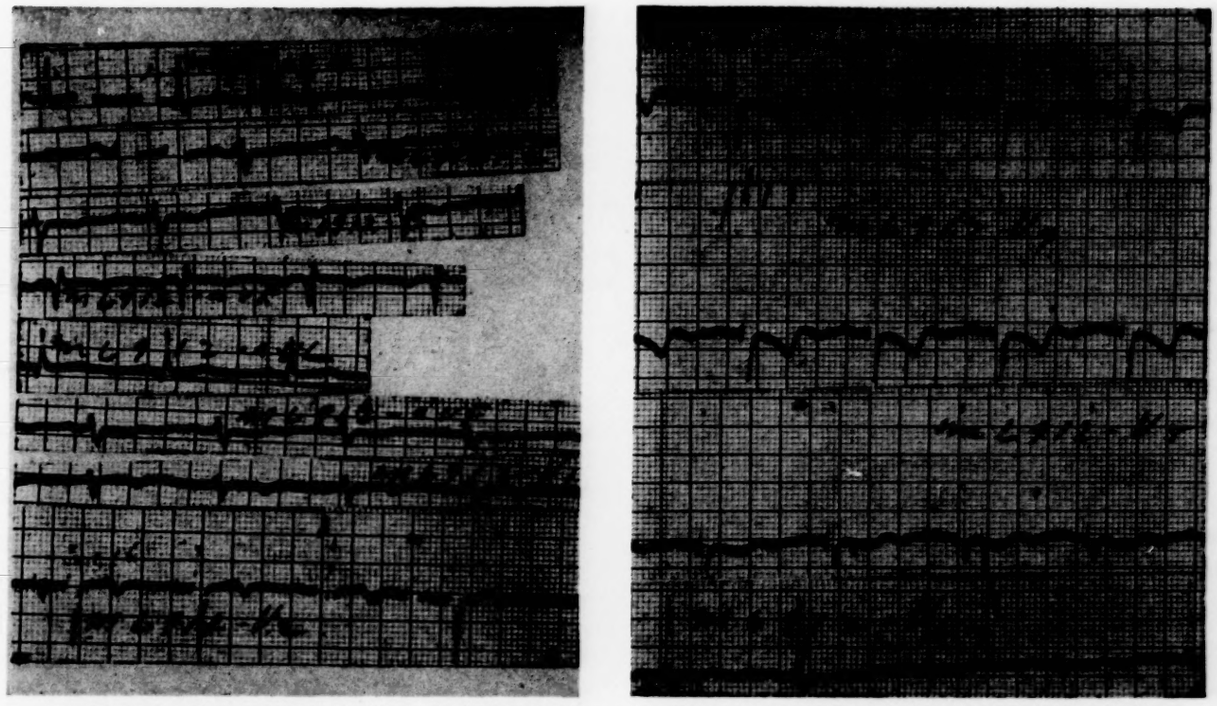

FIG. 1. Electrocardiogram of August 20, 1955. Reading from top to bottom, left, leads I, II, III, AVr, AVl, AVf, V_1 and V_2; right, leads V_3, V_4, V_5 and V_6.

time electrocardiograms showed deepening of the T waves in the precordial leads with extension of the inverted T waves into leads V_5 and V_6. (Figs. 2 and 3.) On the eighth hospital day he began to perspire and had an episode of mild pain in the chest and pain in the left arm which subsided while he was being visited by the house staff. Beginning on the eleventh hospital day, he became restless and had daily bouts of retrosternal burning with radiation to the left arm accompanied with sweating. Nitroglycerine, gr. $\frac{1}{200}$ sublingually, was given once without affording relief. Demerol® was required once to relieve the pain. The patient was nauseated occasionally and vomited a few times. Restlessness and insomnia were difficult to control. On the fourteenth hospital day the patient began running a low-grade fever ranging between 37.5°C. and 38°C. On the fifteenth hospital day he refused to stay at complete bedrest any longer. He was therefore permitted to sit in a chair at the bedside to eat his meals and was also allowed to go to the bathroom for bowel movements. On the nineteenth hospital day an electrocardiogram revealed Q waves in leads V_1 to V_3 and S-T segment elevations in leads V_2 to V_6; these changes were interpreted as being indicative of an acute anteroseptal myocardial infarction. (Fig. 4.) On the twentieth hospital day, because of the agitated and depressed state of the patient, phenobarbital was increased to 60 mg. every six hours, and demerol was used to control the chest pain and allay anxiety. These measures were for the most part ineffectual. On the twenty-first hospital day fine inspiratory rales were heard at the lung bases. On the morning of the twenty-second hospital day the patient was dyspneic and moderately cyanotic. There was an increase in the rales at the lung bases, the heart rate was 120 per minute, and the heart tones were muffled. The patient was given mercuhydrin,® 1 cc. intramuscularly, and aminophylline intravenously. He received one dose of 0.33 mg. of digilanid. Because of ventricular premature contractions, he was given 0.2 gm. of quinidine every eight hours, and received three doses on the twenty-second hospital day. An electrocardiogram taken at this time no longer revealed the previous findings suggestive of acute anteroseptal myocardial infarction. (Fig. 5.) At 7:30 P.M. on the twenty-second hospital day the patient sat up in bed with his face turned upward. His pulse was noted to be irregular. He became very cyanotic, did not utter a cry, and died within a few minutes.

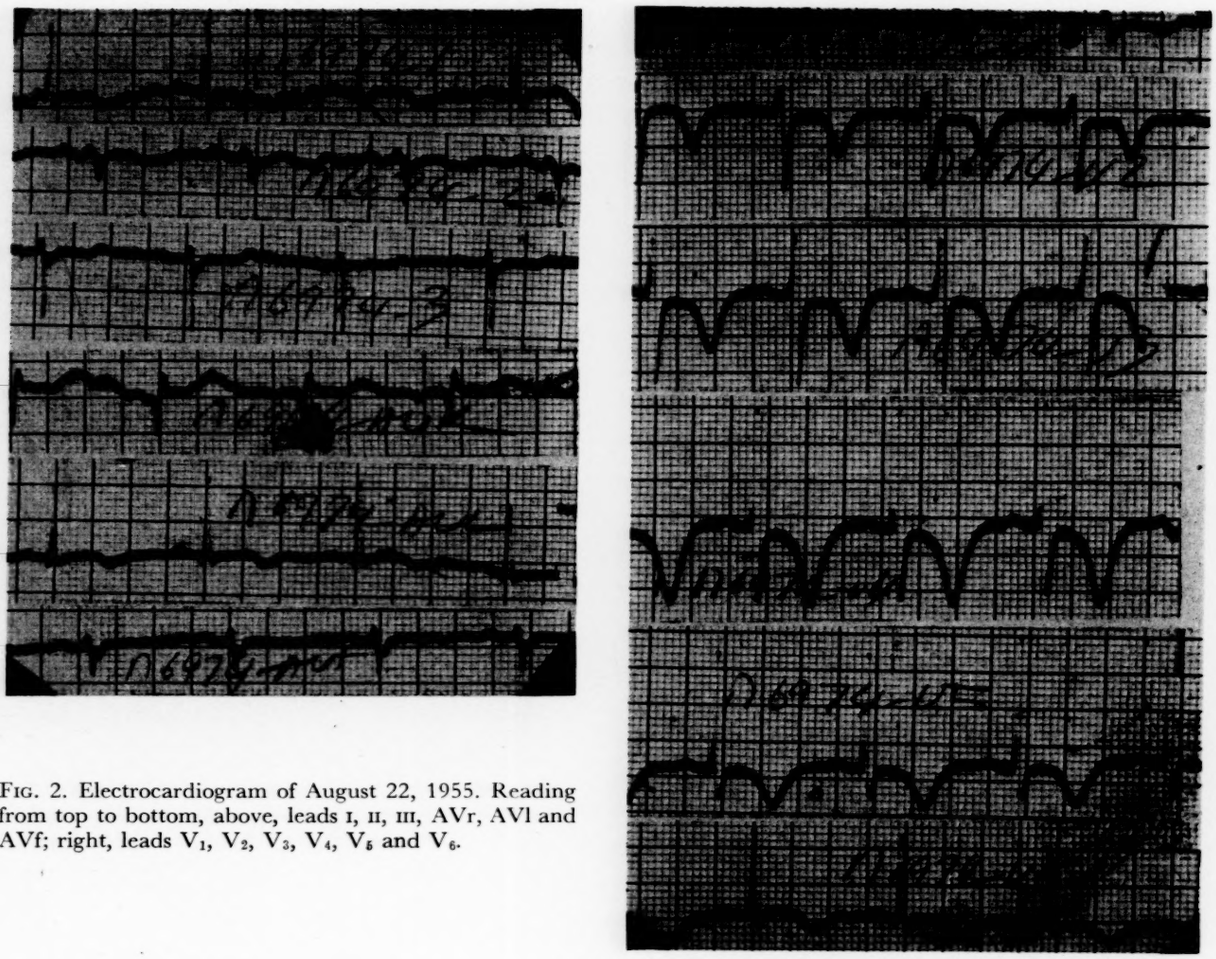

FIG. 2. Electrocardiogram of August 22, 1955. Reading from top to bottom, above, leads I, II, III, AVr, AVl and AVf; right, leads V_1, V_2, V_3, V_4, V_5 and V_6.

CLINICAL DISCUSSION

DR. EDWARD REINHARD: There is one feature in the patient's history, not mentioned in the abstract, that may be of significance. This patient was a traveling salesman who lived in Kansas. The episode of severe, burning, retrosternal pain radiating to the left shoulder and arm accompanied with dyspnea which finally resulted in his hospitalization, began at 1:00 A.M. He was having intercourse at the time the burning pain began. It was specifically stated in the history that during the preceding three years he had had approximately fifty episodes of similar substernal pain. These episodes lasted from one half to one hour, and were at times accompanied with dyspnea. Prior to the episode which brought the patient into the hospital, the pain had not been precipitated by intercourse, exertion, excitement, meals or exposure to cold. These attacks of pain usually occurred after the patient had retired for the night and during the night. Dr. Massie, would you discuss the history in terms of the possibility that the patient had a myocardial infarction. What types of activity usually precipitate myocardial infarction?

DR. EDWARD MASSIE: The actual precipitation of a myocardial infarction may be attended by no exertion at all, and the nocturnal occurrence of infarction is very common. The episode will often wake the patient out of a sound sleep. It would be rather difficult in a brief discussion to present data as to the types of activity which are associated with the gradual precipitation of an infarction. I would assume that at least half of the patients who develop infarctions will develop them during sleep, and the other half will get them in association with actual exertion. Of course, many times a patient may exert and have an initial pain which will subside and then recur severely during the night

DR. REINHARD: What is the mechanism for the

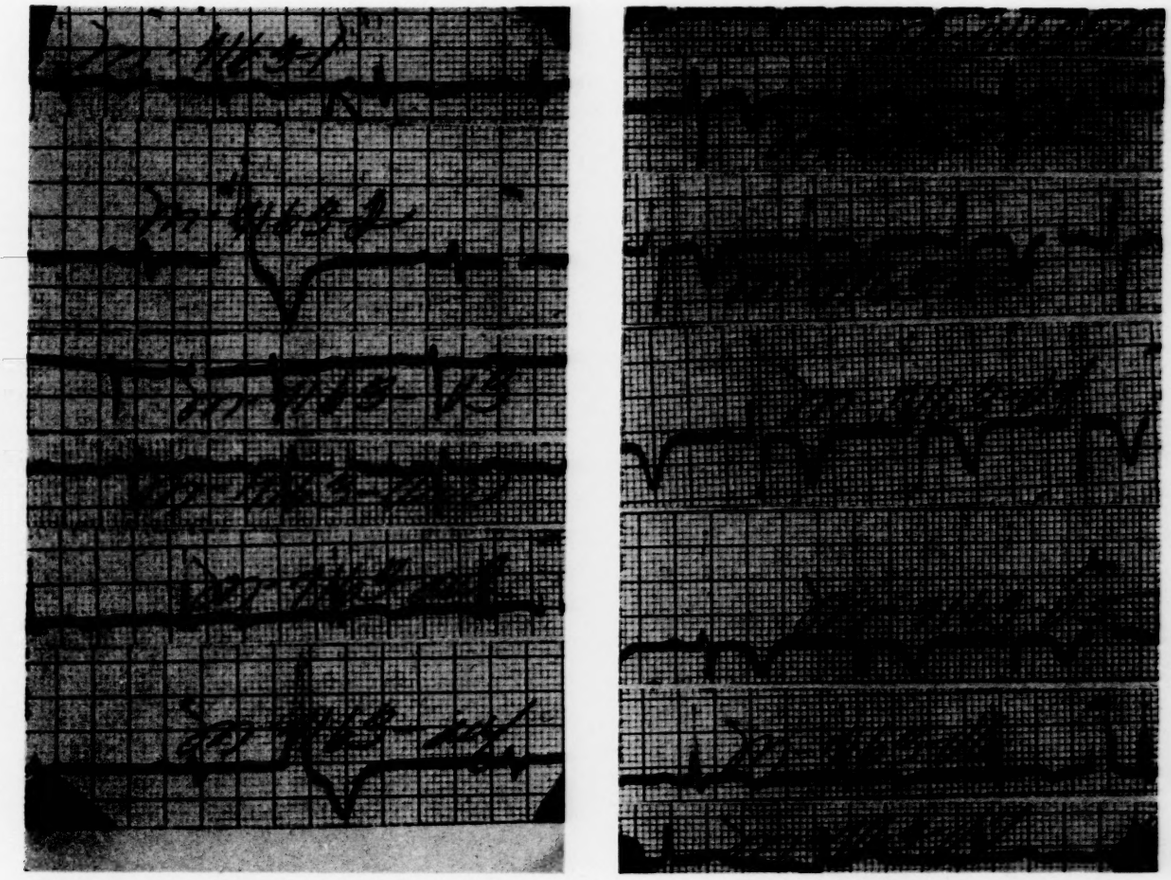

FIG. 3. Electrocardiogram of August 29, 1955. Reading from top to bottom, left, leads I, II, III, AVr, AVl and AVf; right, leads V_1, V_2, V_3, V_4, V_5, V_6 and V_7.

frequent occurrence of infarction during the night when the patient is asleep?

DR. MASSIE: I think the mechanism depends primarily on the lowered cardiac output, the decrease in blood pressure and the associated decrease in coronary blood flow.

DR. HERBERT ZIMMERMAN: About three years ago, Master and Jaffe reviewed this subject. In a large series of patients, they compared the percentage of infarcts occurring while asleep and while awake. They found that if one takes into consideration the amount of time spent sleeping, as many infarcts occur at night as during the day. It has also been pointed out, however, that when the histories of these people are examined, a period of extreme exertion twenty-four to forty-eight hours preceeding the onset of the acute pain is often noted. It has been suggested that the initial stages of the infarction may occur some time before the onset of the actual pain.

DR. REINHARD: It seems, therefore, that sleep, when a person is at complete rest, certainly is no protection against myocardial infarction. I would like to focus your attention on the nature of the substernal pain. The patient consistently described it as a burning pain. Dr. Smith, would you discuss the character of the pain usually noted in association with myocardial infarction, and tell us your ideas on the mechanism of the production of this pain?

DR. JOHN SMITH: In analyzing any symptom that may be construed as cardiac pain it is well to recall that the myocardium, itself, is presumably painless. Therefore, the pain of myocardial disease arises from some other source. It was stated years ago that the pain of angina pectoris may arise from distention of the adventitia of the aorta or of the coronary arteries. This concept has been rather difficult to analyze experimentally, and the evidence has not been wholly convincing. More recently this problem has been brought up again by the suggestion that pain may arise from distention of the pulmonary artery. Some workers have suggested esophageal spasm as the source of cardiac pain. I think most

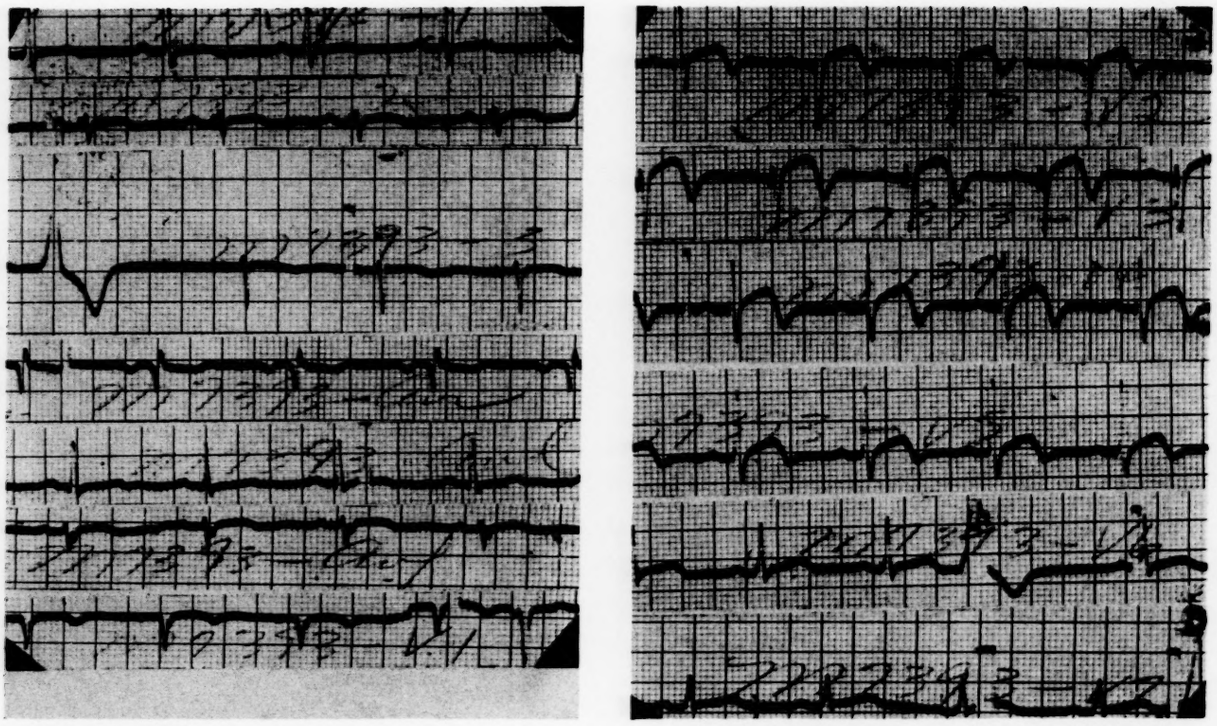

FIG. 4. Electrocardiogram of September 6, 1955. Reading from top to bottom, left, leads I, II, III, AVr, AVl, AVf and V_1; right, leads V_2, V_3, V_4, V_5, V_6 and V_7.

people believe that the pain of angina or of myocardial infarction arises from ischemia of the heart muscle, impulses from which play upon the spinal cord and on reflexogenic mechanisms at different sites in sensitive structures in the chest. It has been suggested that contraction of the internal intercostal muscles may give rise to the immediate sensation of discomfort due to the widespread stimuli received by the spinal cord. The pain noted by this patient is a little different from the usual type of cardiac pain and would suggest to me an element of vasodilatation somewhere in the structures of the chest wall rather than predominant muscle spasm. Burning pain in angina pectoris has been described and is accepted as one of the variants of such symptomatology. It is interesting that this pain usually occurred at night. I believe that you will find that patients who have angina pectoris at night often have aortic insufficiency, or a process which involves the mouths of the coronary arteries. I would raise the question of aortitis with involvement of the coronaries in this case, although clinical proof would be difficult to establish. Among the other pains to be considered in this situation is dorsal root pain which often occurs at night. One must also be alert to the possibility of diaphragmatic hernia, which can produce pain so resembling heart pain as to be indistinguishable. Mediastinitis and disease of the upper abdominal organs, such as the pancreas, may occasionally produce similar pain. There is very little evidence for the latter here, and it seems to me that this patient's pain was probably cardiac in origin.

DR. REINHARD: The important point to emphasize is that burning is not the usual description for anginal pain or pain of cardiac origin, but it certainly can occur. One other point in the history deserves comment. Between the ages of nine and seventeen the patient had approximately thirteen episodes of polyarthritis with pain and swelling of various joints. Each of these episodes cleared up without residual joint deformity of cardiac sequelae. Dr. Glaser, the absence of any evidence of valvular disease after thirteen attacks of rheumatic fever would, I believe, be unusual but certainly not unheard of. The absence of joint deformity is against rheumatoid arthritis in childhood, so-called Still's disease. Would you comment and tell us which of these diagnoses seems more likely to you?

DR. ROBERT GLASER: Recurrent attacks of rheumatic fever without the ultimate development of valvular cardiac disease is unusual, but

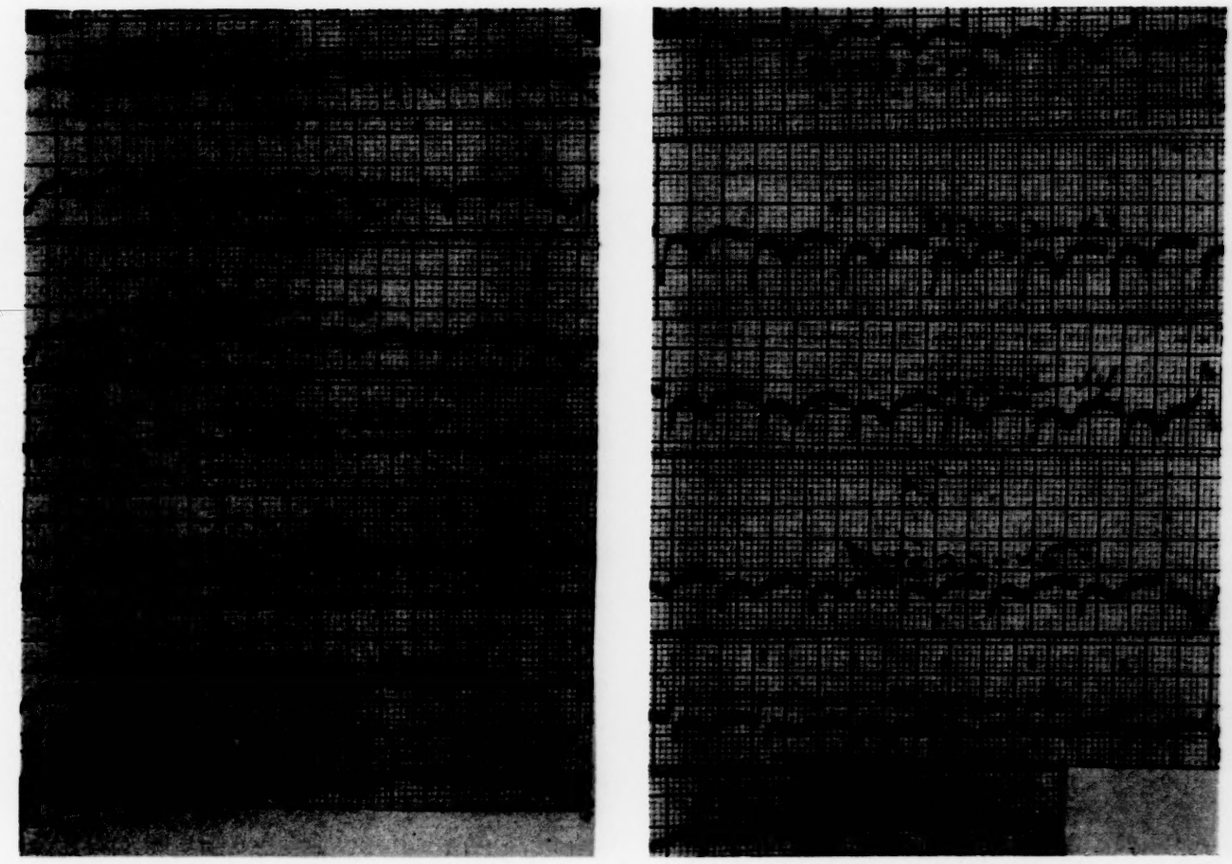

FIG. 5. Electrocardiogram of September 10, 1955. Reading from top to bottom, left, leads, I, II, III, AVr, AVl, AVf and V_1; right, leads V_2, V_3, V_4, V_5, V_6 and V_7.

such cases do occur. I know of one family in which six members have at various times had recurrent attacks of acute rheumatic fever with joint manifestations, and in none of these patients, followed over many years, has cardiac disease developed. I have had very little experience with Still's disease, but I agree with you that, in general, one would have expected deformity if the arthritic attacks had been due to Still's disease. I would presume that this man did have rheumatic fever in his youth but was fortunate enough in having had the inflammatory process confined to the joints which characteristically are left free of permanent damage.

DR. REINHARD: I believe that is correct. In general, in Still's disease the degree of joint deformity and actual bone injury is considerably greater than in rheumatoid arthritis in adults. After several attacks one would certainly have expected some deformities.

DR. BERCU: I believe it is interesting that the patient had two unusual findings in addition to the obvious cardiac manifestations. One is the history of recurrent joint disease and the other is that he had calcification of the intervertebral discs. In ochronosis, patients who have arthritis often have calcification of the intervertebral discs, and the majority die of arteriosclerotic heart disease. I believe one should at least consider ochronosis as another cause of the arthritis.

DR. REINHARD: Perhaps a few words should be said about the patient's personality. He was a dynamic, hard-working salesman. The day before admission to Barnes Hospital, he drove to St. Louis from Davenport, Iowa, stopping only to drink milk. He ate nothing throughout the entire trip but did have several glasses of milk. We do not know why he drank the milk. There was no statement that he had any epigastric distress or burning, but he certainly did not eat anything. When he arrived in St. Louis, he had a sandwich and a glass of beer. He then had several highballs in his hotel room. He was a very heavy smoker, apparently a fairly heavy drinker. After admission to the hospital, it was almost impossible to keep him in bed. He was

allowed to get up, even though there was considerable evidence that he had a myocardial infarction, since it was quite obvious that he was going to get up out of bed whether he was allowed to or not. While in the hospital, it was noted that he was very tense. He slept very poorly, even when he did not have any pain. Dr. Graham, does this sound like the type of person whom you would expect to be coronary prone, or is there such a thing as the coronary personality?

DR. DAVID GRAHAM: I do not believe that there is general agreement that there is a particular kind of personality in those who are prone to coronary artery disease. That is not to say that it may not be true, but that satisfactory evidence has not yet been adduced.

DR. REINHARD: Would you comment on one other feature. How do you handle such patients? Do you give in and permit them to be up and around, or do you sedate them heavily and try to keep them in bed?

DR. GRAHAM: It seems that the best thing to do with persons who insist on getting up is to let them get up. If you try to restrain them physically or force them to remain in bed they simply become aggitated, and you get into a very unfortunate battle between the physician and the patient, which I think is much worse than a certain amount of exercise. I believe it is usually possible to work out a compromise if there is good will on both sides so that the patient is not, in fact, exercising so violently that he is likely to do serious damage to his heart.

DR. REINHARD: That was apparently done in this case. Dr. Powers, would you review the chest films?

DR. WILLIAM POWERS: The chest films were taken on the sixteenth day after hospital admission. The bony structures appeared normal. The cardiac silhouette was normal. Aside from some protuberance of the aortic arch, there was nothing characteristic of enlargement of the aorta. The ascending aorta appeared completely normal. The calcification of the intervertebral disc, as Dr. Bercu mentioned, was seen between D10 and D11 and possibly one intervertebral disc space higher. There was no evidence of hiatus hernia, although no specific examination was carried out with a barium swallow or gastric fill-up.

DR. REINHARD: Dr. Massie, would you discuss the electrocardiograms?

DR. MASSIE: We have an excellent electrocardiographic demonstration in this case. The first record was taken on August 20, 1955, and you will observe that the standard leads are not significantly abnormal. (Fig. 1.) Note also the QR pattern in lead aVR; later on you will see that the R waves in this lead increase in voltage. The T waves are inverted in leads V_1 through V_4 and diphasic in V_5 and V_6. With the history we have just heard and with the inverted T waves in the precordial leads, this record may be considered as indicating the presence of anterior myocardial ischemia and is compatible with coronary insufficiency. Also notice that there is high voltage in lead V_5 amounting to almost 25 mm. If we had a deeper S wave in V_1, we would suggest that this patient had left ventricular enlargement. We cannot make such a diagnosis, however, and as a matter of fact, the x-ray also does not indicate its presence. The second record was taken two days later on August 22nd. (Fig. 2.) In lead I, the T waves have now become inverted although lead II has not changed significantly. In aVR the QR configuration has remained the same, but the T waves have become inverted in aVL. In leads V_1 through V_6 extensive inversion of the T waves has occurred. It appears to me that there is more evidence of anterior myocardial ischemia, but such a change also could be compatible with some evidence of infarction, with the necrosis of muscle not being quite transmural in extent.

On the next record of August 29th, (Fig. 3) one ventricular extrasystole was encountered and the only change to be noted is the fact that the T waves are generally slightly less inverted in the V leads and actually are practically upright in V_6 and completely upright in V_7. It would appear, perhaps, that this record is indicative of slightly less ischemia than the previous one.

DR. REINHARD: Dr. Massie, the record of August 29th was taken nine days after the precipitating attack of pain that brought the patient to the hospital and the subsequent record was taken almost nine days later. If you assume that the original pain was due to an actual infarction, would you not have expected changes by this time?

DR. MASSIE: We do see some change in the records with increase in the electrocardiographic evidence of myocardial ischemia at first, and a shade less on the record just discussed. One could say that this man was in the throes of progressive coronary insufficiency which was

evoluting into a definite myocardial infarction. Perhaps at this particular stage of his disease, had he been more cooperative and quiet he might have had the chance of reversing some of the myocardial ischemia and injury. It appears to me that it is always important to hospitalize the patient, giving him prompt care and treating him with all means at our disposal, including anticoagulation.

The next record was taken about a week later on September 6th. (Fig. 4.) Now distinct and significant changes have appeared. The T waves tend to be slightly upright in leads I and aVL whereas there was some tendency toward inversion before. Q waves are definitely present in V_1 and V_2 with a Q and a small R wave in V_3. The S-T segments are somewhat elevated in leads V_2, V_3, V_4 and V_5. The classic picture of an anteroseptal myocardial infarction has now appeared.

The final record taken on September 10th shows a very distinct and significant group of changes. (Fig. 5.) The T waves have become inverted in I, as have those in aVL. The voltage is generally lower in the standard and unipolar extremity leads. The R waves are smaller in lead aVF than before. The voltage of the R waves has become surprisingly higher in V_2, V_3 and V_4. The appearance of these high R waves strongly points to the development of a second myocardial infarction and this time the location is in the posterior myocardium. We are faced with the following question. Why should necrosis of muscle in the anterior myocardium which produced Q waves as on the record of September 6th, suddenly, in the record taken a few days later, no longer produce Q waves? Certainly there has been inadequate time for recovery to occur to account for disappearance of the Q wave. It must be remembered that the depolarization forces in the posterior wall are normally negative with respect to an electrode located adjacent to the anterior surface, that is on the anterior chest wall. They, therefore counterbalance, to a large extent, the positive voltages which could be recorded by the exploring electrode on the chest wall. When there is a large area of necrosis on the posterior wall, the opposing forces in question are reduced. This change results in an increase in the height of the R deflections in the leads from the right side of the precordium. This record would suggest that such a mechanism occurred in this patient. However, there is another possible lesion to be considered. The R waves are higher in lead aVR and there is some evidence of clockwise rotation with S waves appearing more distinctly in the lateral precordial leads, namely V_6 and V_7. These changes may support the possibility of a pulmonary embolus, but I believe that less likely than the development of the complicating posterior myocardial infarction.

DR. REINHARD: By way of summary, Dr. Massie has pointed out that this patient, by the end of the second week in the hospital, developed unequivocal electrocardiographic evidence, or at least very convincing electrocardiographic evidence, of an anteroseptal myocardial infarction. The chief problem which arose as to the diagnosis in this case centers around the fact that between the record of September 6th and the last one, there was a rather striking change in the direction of greater normality with the reappearance of R waves in V_2, V_3 and V_4. Dr. Massie, in the official interpretation of the electrocardiogram it was suggested that these changes might be due to the superimposition of a posterior myocardial infarction, such as you have mentioned, but it was also stated that this might be due to differences in electrode placement. Is this really true?

DR. MASSIE: Yes, that is conceivable. If the technician is inexperienced, it is quite possible that the electrode might be placed in the proper position for lead V_1 but through carelessness or lack of knowledge the electrode to obtain lead V_2, instead of being placed in the fourth intercostal space at the left sternal border, might have been moved too far over to the left side of the chest and possibly placed in the position for what is actually lead V_4. Such an error would give a high R wave in what is presumed to be lead V_2 and would throw our deductions completely awry. We would then miss the fact that the Q waves are still present in leads V_2 and V_3. However, I do not believe that this record was taken so incorrectly nor do I consider that possibility at all likely.

DR. REINHARD: Dr. Zimmerman, I believe you saw these electrocardiograms when the patient was still in the hospital. Do you have anything to add to what Dr. Massie has stated? We have at the moment three possibilities: (1) that the electrodes may have been misplaced, which Dr. Massie believes is unlikely; (2) that the patient might have suffered a superimposed posterior myocardial infarction; and (3) the possibility of a superimposed

pulmonary embolus. Would you like to defend any of these possibilities or add any others?

DR. ZIMMERMAN: I believe it should be pointed out that a deep S wave in I and a lower R wave in III have suddenly appeared. The occurrence of S-1, Q-3 pattern is typical of acute pulmonary embolism. That, plus the clockwise rotation with deeper S waves in V_5, V_6 and V_7 might make one favor a diagnosis of superimposed pulmonary embolus. There is insufficient evidence to defend any of these suggestions strongly but I believe a superimposed posterior infarction is a very likely possibility.

DR. REINHARD: Dr. Kenamore, is it possible that the patient's numerous episodes of substernal burning as well as the terminal events might have been due to some primary gastrointestinal lesion such as a hiatus hernia with peptic esophagitis. I might state that this diagnosis was seriously entertained when the patient was first admitted to the hospital. Could such a lesion account for the course of events in this case?

DR. BRUCE KENAMORE: I do not believe so. It is difficult to account for the electrocardiographic changes on the basis of esophageal disease. The pain could be so explained. Experimentally, a good deal has been done in an attempt to reproduce the pain and provoke the electrocardiographic changes of infarction by stimulation at various levels of the esophagus. For the most part these experiments produced only occasional aberrations in the electrocardiogram. Electrocardiographic changes compatible with myocardial ischemia have not been provoked even in patients who are known to have coronary insufficiency.

DR. REINHARD: Is it not true that with peptic esophagitis leading to perforation of the lower esophagus and the production of mediastinitis, patients occasionally die from cardiac diseases?

DR. KENAMORE: I believe they probably all do. I think it was shown some years ago that in dogs the mechanism of death due to mediastinitis is cardiac tamponade. I do not know what kind of electrocardiographic changes that would produce. I assume they would be those of pericarditis.

DR. REINHARD: I believe that the patient had an anteroseptal myocardial infarction. I believe that the patient then had either (1) a pulmonary embolus, or (2) a posterior myocardial infarction to account for the electrocardiographic changes in the last tracing. There is an outside possibility that the patient might have ruptured a ventricular aneurysm as a terminal event, although there is little positive evidence for this diagnosis.

PATHOLOGIC DISCUSSION

DR. ROBERT C. AHLVIN: The most pertinent anatomic findings concerned the heart of this moderately obese white man. Although 700 cc. of fluid and clotted blood distended the pericardial sac, its serosal surfaces were smooth. There was a 2 cm. rupture of the lateral wall of the left ventricle. A large mural thrombus was found on the endocardium of the left ventricle over the anterior portion of the septum and extended along the distal two-thirds of the anterior wall. Under the thrombus, the ventricular wall was very thin, mottled, soft and yellow, particularly in the apical portion. This discoloration and softness extended anteriorly to the lateral wall of the left ventricle and superiorly to include the site of rupture. Valves were grossly normal, although microscopically small muscular arteries were present in the mitral valve leaflets, possibly representing stigmata of a previous inflammatory episode. The chordae tendinae were normal. Severe arteriosclerosis of the coronary arteries was generalized and had reduced the lumen of the anterior descending branch of the left coronary artery to a slit. Two centimeters from its origin the circumflex branch of the left coronary artery was occluded by an organizing thrombus. The right coronary artery supplied posterior aspects of the septum and left ventricular wall as well as right ventricle.

Over the area of thinning near the apex of the anterior wall of the left ventricle, little evidence of organization could be demonstrated within the mural thrombus. (Fig. 6, lower part.) Myocardial cytoplasm adjacent to the endocardium is very eosinophilic and striations have been lost. Only nuclei of monocytes and other inflammatory cells are visible in this area, as those of myocardial cells have disappeared. A zone of highly vascular proliferating granulation tissue separates this zone of dead muscle from the myocardium below it. (Fig. 7.) Hemosiderin-laden macrophages are rarely encountered. The rest of the ventricular wall in this area is composed of swollen, pale-staining myocardial fibers with normal appearing myocardial nuclei. (Fig. 6, upper portion.) This type of abnormal appearing myocardial fibers is frequently seen about myocardial infarcts. This lesion correlates with the history of the first

FIG. 6. This microsection (hematoxylin and eosin paraffin) was prepared from the ventricular wall in the region of the oldest infarct. (× 150 approximately.)

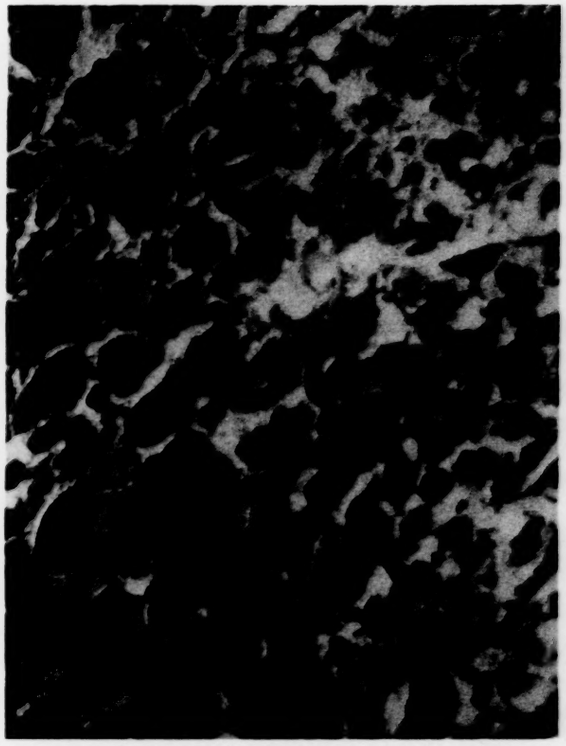

FIG. 7. Proliferating granulation tissue (upper right) is shown at the edge of the infarct depicted in Figure 6. (× 400.)

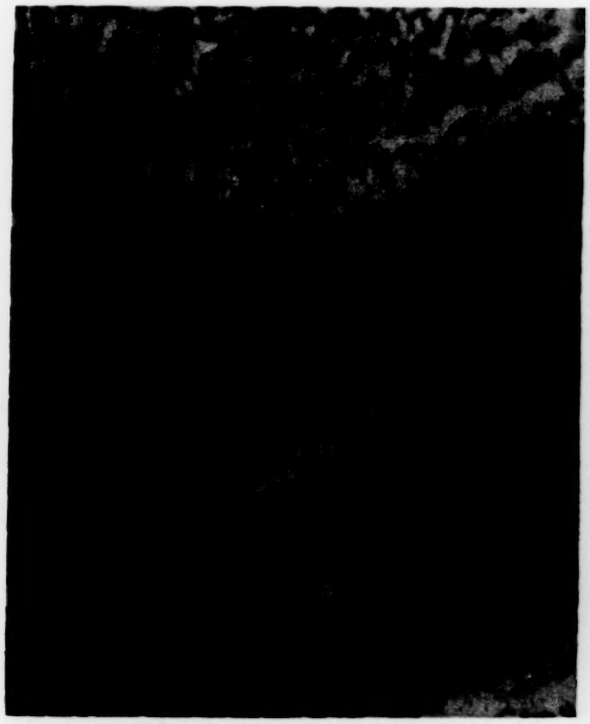

FIG. 8. Appearance of myocardium adjacent to the point of rupture. (× 20.)

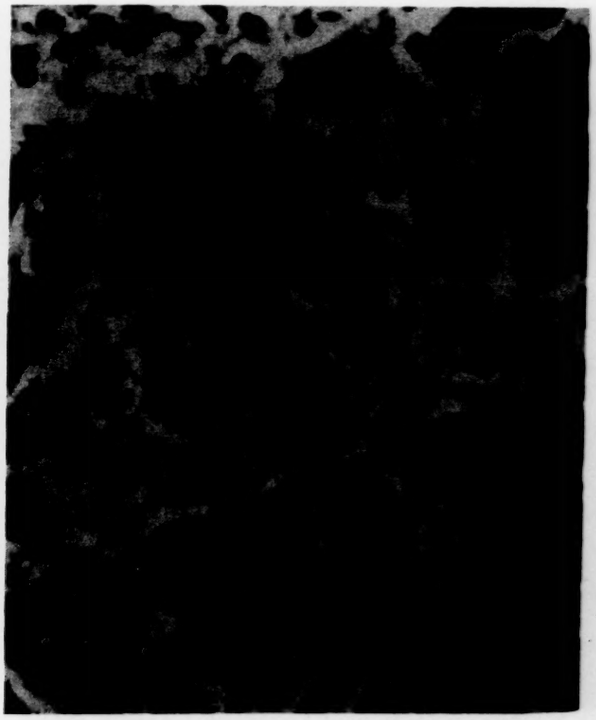

FIG. 9. Polymorphonuclear leukocytes (upper left) can be seen in this higher magnification (× 400) taken from the field in Figure 8.

infarction experienced by the patient (twenty days).

In a section from the lateral wall of the left ventricle adjacent to the point of rupture the astriate cytoplasm of the myocardial fibers is intensely stained and almost homogeneous, and the fibers are widely separated by an infiltrate of inflammatory cells, predominantly polymorphonuclear leukocytes. (Figs. 8 and 9.) Myocardial nuclei have disappeared. This lesion represents a very recent infarct, probably just a few days old, involving the entire ventricular wall. Fat stains on frozen sections from this area reveals the marked accumulation of stainable lipid within damaged myocardial fibers.

The lungs were heavy (over 2,000 gm.). A large amount of frothy fluid escaped from the cut surface of the bright red parenchyma, but areas of consolidation could not be demonstrated.

An interesting point in this case is the distribution of vascular disease in the small arteries. The kidneys were of normal size; their capsules were smooth. Microscopically, most of the arteries in the kidney were normal, but in the walls of a few arterioles and small muscular arteries small amounts of subendothelial hyaline material had been deposited. In contrast, marked subendothelial hyalinization of the walls of the small arteries and arterioles of the pancreas and liver was a prominent feature.

Final Anatomic Diagnoses: Arteriosclerosis of the coronary arteries, advanced, with almost complete occlusion of the anterior descending branch of the left coronary artery by arteriosclerotic plaques; healing infarct of the myocardium involving the apex and anterior wall of the left ventricle with mural thrombus in the left ventricle; occlusion of the circumflex branch of the left coronary artery by thrombus; recent infarct of the myocardium involving the anterior and lateral walls of the left ventricle and the anterior portion of the interventricular septum; rupture of the lateral wall of the left ventricle; hemopericardium; congestion of the liver, spleen and lungs; arteriosclerosis of the aorta, basilar, internal carotid and vertebral arteries, moderate; arteriolar nephrosclerosis minimal; arteriolar sclerosis in the pancreas and liver, marked; hypertrophy and dilatation of the heart, moderate (530 gm.); vascularization of the mitral valve leaflet.

Dr. W. Stanley Hartroft: This autopsy provides material for a study of myocardial infarctions of various ages, all of which are related to the electrocardiographic changes. Sections of the heart even demonstrated encroachment of one infarcted area on another. (Fig. 6.) The lack of inflammatory reaction at the edge of the recent infarct is a manifestation of the devitalization of tissue in the adjacent, older lesion.

I want to speak briefly about the epidemiologic aspects of myocardial infarction. Dr. Kyu Taik Lee and Dr. Thomas* in our department found that the incidence of myocardial infarction in Barnes Hospital autopsy population prior to 1940 was twice as frequent in men as in women, in agreement with many other reports published about that time. But during the past fifteen years myocardial infarction in patients coming to autopsy from this hospital has been as frequently encountered in women as in men. Drs. Lee and Thomas failed to demonstrate that hypertension played any etiologic role in myocardial infarction. In their series the kidneys of all patients with known hypertensive disease weighed 100 gm. less than those of patients without hypertensive disease. But in patients who died with acute myocardial infarction, the kidneys weighed but little less than those of the non-hypertensive control groups and the kidneys of a series of patients dying of cancer weighed essentially the same as those dying of myocardial infarction. Therefore, hypertension in these individuals could not be regarded as a factor of etiologic importance in the production of myocardial infarction.

Dr. Thomas and Dr. Lee also compared the incidence of myocardial rupture in patients in this hospital who died with myocardial infarction before the institution of anticoagulant therapy and since that time. The incidence of rupture before the use of anticoagulants was just as great as in patients with myocardial infarction who had been treated with these drugs.

Dr. Glaser: Dr. Reinhard, I think this case is of interest in one regard. Some years ago Dr. Paul White compared the incidence of rupture of the ventricle in patients with myocardial infarction hospitalized in a general hospital with a similar group in a psychiatric hospital and found the incidence much greater in the psy-

* Myocardial infarction: changing sex ratio and other factors. An epidemiological study of acute myocardial infarction based on the experiences of Barnes Hospital for 45 years. Lee, K. T. and Thomas, W. A. To be published in *Arch. Int. Med.*, 1956.

chiatric patients, presumably because of their unwillingness or inability to cooperate. I think that here one might well assume that this man's unwillingness to cooperate and to rest might have been a factor contributing to the outcome.

DR. MASSIE: Dr. Hartroft, do you have statistics available for the ages of fifty or under? I know, as you said, that the women are from the upper age group. Am I correct in assuming that although the ratio was 1 : 1, the women were older, because under fifty I would think our ratio was something like four men to one woman, perhaps five to one clinically. Under forty we see many men with myocardial infarction, but I can only recall seeing perhaps twelve women.

DR. HARTROFT: The average age at which myocardial infarction developed in men before 1940 is the same as that of males who died of myocardial infarction since. There has been an increase since 1940 in the number of women in the sixth and seventh decades of life who have come to autopsy at this hospital, as a result of myocardial infarction.

DR. HENRY A. SCHROEDER: Would you mind if I do not let that statement about hypertension and its effects on myocardial infarction go by without comment. Of course, it is obvious that one can have hypertension for a good many years without getting any demonstrable changes in the kidneys in man.

DR. HARTROFT: If hypertension were an etiologic factor in the production of myocardial infarction in these patients, it must have been present for a number of years without being associated with a decrease in the size of the kidneys. But in the entire group of hypertensive patients selected for comparable age, weight and sex, hypertension *was* associated with a decrease in kidney weight. These two facts make it seem very unlikely to us that hypertension could have been present to a significant degree for very long in many of the patients dying of myocardial infarction.

DR. MASSIE: This is a difficult question to answer; here is a patient who had had no anticoagulant therapy and had a succession of infarctions and ventricular rupture. Looking back can the pathologist say that we could have done something with anticoagulant therapy? Would this patient have improved if he had had anticoagulant therapy, or were his coronary vessels so narrowed that anticoagulant therapy would have been of no avail?

DR. REINHARD: This patient had anticoagulant therapy but only during the last twenty-four hours of life.

DR. HARTROFT: I believe that in this case anticoagulant therapy could not have done nearly as much as complete rest. Dr. Massie stated previously that it is most important to keep these patients at rest and at complete rest if possible. Had the latter been achieved in the case of this patient, it surely would have helped the severely damaged myocardium repair and perhaps have prevented rupture.

Acknowledgment: Illustrations were made by the Department of Illustrations, Washington University School of Medicine. Photomicrographs were prepared by the Department of Pathology.

Case Reports

Cryoproteinemia: An Immunologic Phenomenon?*

Electrophoretic Analysis of Serum Proteins of a Patient with Cold Allergy

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In 1933 Wintrobe and Buell[25] described the presence of a cold-precipitable protein in the serum of a patient with multiple myeloma. Von Bornsdorff et al.,[3b] and Holmberg and Grönwall[7a] reported further studies on the physical characteristics of this unusual serum moiety. Later, Lerner and Greenberg[12] investigated a similar substance. It was derived from a patient who exhibited an abnormal reaction to low temperatures. In this case and others the disorder was manifested by purpura, urticaria, Raynaud's phenomena, thrombosis of the retinal vessels, deafness and arthritis.[1,11,13]

This protein had several unusual properties. With respect to ultraviolet absorption spectrum and nitrogen content, it resembled gamma globulins; unlike these, it was soluble in water and insoluble in dilute salt solutions. The molecular weight (190,000) and the viscosity were both greater than that of normal human gamma globulin.[12]

Using the moving boundary method of electrophoresis, the mobility of "purified" cryoprotein has been determined by several investigators in solutions of relatively high hydrogen ion concentration.[1,7a,12] Its relations to the other serum proteins have not been intensively studied. Recently, however, Osserman and Lawlor,[15a] using both boundary and filter paper electrophoresis, have identified as a homogeneous gamma globulin the cryoprotein obtained from one of their cases of multiple myeloma.

This study examines the electrophoretic distribution of serum proteins in a patient with cold allergy. An analysis was performed before and after separation of the cold-precipitable component. Subsequently, the patient was treated with cortisone for six days. At the end of this time he no longer reacted abnormally to cold; the serum, concomitantly, yielded no precipitate in the refrigerator. The electrophoretic pattern derived from the serum after treatment with cortisone is analyzed and a comparison is made with the patient's pretreatment serum proteins and those in normal pooled serum. The resemblance of cryoproteins to immune bodies is discussed.

CASE REPORT†

A. E. A. (R. N. 100,619), a forty-two year old single white male laborer, was admitted to the Veterans Administration Hospital in Portland, Oregon, on March 7, 1955. He complained of severe itching and "breaking out" after exposure to cold.

He had believed he was in good general health until about eleven years prior to admission (1944). At that time, while stationed in Alaska with the Armed Forces, he noted the presence of generalized pruritus, always after exposure to cold for ten to fifteen minutes; an eruption consisting of pea-sized welts with pale centers developed on the arms, legs and the dorsa of the feet. Return to the warmer temperatures of a heated room resulted in immediate subjective improvement and fading of the urticaria after fifteen to twenty minutes.

Physical examination at the time of admission revealed a well developed, well nourished white man in apparently good health. No abnormalities were detected.

† The details of the clinical aspects of this case will be described in a subsequent communication.[26]

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Laboratory data were as follows: hemoglobin, 15.4 gm.; erythrocytes, 5.07 million; white blood count, 6,900; neutrophils, 46 per cent; stab forms, 2 per cent; lymphocytes, 44 per cent; monocytes, 5 per cent; eosinophils, 3 per cent. Erythrocyte sedimentation rate was 3 mm./hr. Bone marrow aspiration revealed no abnormalities in morphology, number or type of cells present. No myeloma cells were seen. Urinalysis revealed the following data: urine, straw yellow in color; specific gravity, 1.025; reaction, acid; albumin, Bence-Jones protein and sugar were not present; occasional white cells were seen per high power field. Total protein was 6.9 gm. per cent (method of Weichselbaum[24]) after precipitation of cryoprotein. Skeletal x-rays indicated no abnormalities.

Throughout the period of hospitalization (March 7 to June 13, 1955) the patient was afebrile and asymptomatic except when exposed to cold. This was accomplished by allowing him to go outdoors in scanty clothing when the temperature was 0°C. or less, and by letting him stand in a room maintained between 0°F. and 10°F. Under these conditions pruritus, urticaria and mottling of the skin always developed. However, applications of ice cubes or water at 0°C. repeatedly resulted only in erythema over the areas of contact.

Oral administration of cortisone was begun on April 13th and was discontinued six days later when a total of 200 mg. had been administered. The patient was then exposed to the cold room at a temperature between 0°F. and 10°F. After fifteen minutes he complained of severe chilling, but not of pruritus. No urticaria could be observed; a slight mottling appeared on the back. Blood was drawn at this time and tested for cryoprotein, but none was detected. The electrophoretic pattern described by the serum indicated that the abnormal protein fraction previously present had disappeared.

MATERIALS AND METHODS

Serum. All blood samples analyzed in this investigation were drawn from the veins of the arms. In order to prevent precipitation of cryoprotein with the blood clot, sterile syringes and needles warmed to 37 to 40°C. were employed. Immediately following withdrawal the blood was placed in a sterile test tube and tightly covered. After incubation at 37 to 40°C. overnight in a constant temperature oven the serum was decanted into another sterile test tube and placed in a refrigerator.

Purification of Cryoprotein. The patient's whole serum was allowed to stand at 5°C. for three days. At the end of that time a heavy, white, flocculent precipitate could be observed throughout the serum. After centrifugation for fifteen minutes at 0°C. the supernatant serum was decanted. The precipitate was redissolved in 0.9 per cent saline solution at 37°C. and then replaced in the refrigerator at 5°C. for three days. Again a white, flocculent precipitate appeared which could be separated from the supernatant fluid by centrifugation. Three successive precipitations and solutions in saline were carried out before electrophoresis of the "purified" material was performed.

Pooled Serum. Pooled serum was utilized for comparison with the serum under study. The venous blood of apparently normal subjects was allowed to clot, and the serum was decanted into a sterile test tube. The samples of several patients were mixed together and then quickly frozen to −10°C. When ready for analysis the pooled serum was allowed to come to room temperature slowly.

Electrophoresis. Paper electrophoresis was performed with a Spinco Model-R apparatus and the stained, dry strips were evaluated with the Spinco Analytrol recording scanner and integrator. Each run was for a period of sixteen hours at room temperature (27°C.). Undiluted serum or cryoprotein, 10 lambda (0.010 ml.), in 0.9 per cent saline solution were placed on Whatman 3 MM paper using a sodium diethylbarbiturate buffer of pH 8.6 and an ionic strength of 0.75. A current of 5 ma. was employed throughout.

Protein Staining. The staining procedure was as follows. The oven-dried strips were placed in a rack and covered with protein staining dye solution (0.10 gm. bromphenol blue and 50.0 gm. zinc sulphate ($7H_2O$) diluted to 1 L. with 5 per cent acetic acid) at room temperature for six hours. They were then transferred to a rinse bath of 5 per cent acetic acid at room temperature for six minutes, following which the strips were transferred to another bath containing a similar solution of acetic acid for an additional six minutes. The strips were placed in a solution of 3.0 gm. sodium acetate ($3H_2O$) diluted to 1 L. with 5 per cent acetic acid for six minutes. They were then removed from the bath and the excess fluid removed by blotting the strips between two sheets of clean blotting paper. They were returned to the rack and placed in an oven at 120°C. for fifteen minutes. The strips then were ready for the analyzer and integrator after standing in the open air for thirty minutes or more.

Analysis and Integration. The analyzer and scanner records on graph paper with ink pens. The greater the density of the stain, which is proportional to the concentration of the protein at a given location, the greater the deviation of the pen in an upward direction. At the same time that the strip is analyzed an integrator sums the area under the inscribed curve, and a relative estimate of the quantities present is obtained. If a protein solution of known concentration is analyzed and the area under the curve is integrated, a proportional relationship between the number of units expressing the area under the curve and the concentration of protein in the solution may be obtained.[3a]

The total protein in the serum of the patient under study, from which cryoprotein had been separated,

TABLE I

QUANTITATIVE ESTIMATIONS OF THE ELECTROPHORETIC PROTEIN DISTRIBUTIONS (GM. PER 100 ML. OF SOLUTION)

	Total Protein	Albumin	Globulins				
			Alpha-1	Alpha-2	Beta	Gamma	Gamma Plus "T"
Normal pooled human serum	7.05	3.92	0.19	0.97	0.86	1.11	.....
Patient's whole serum (serum with cryoprotein)	8.19	4.38	0.38	0.92	1.08	....	1.49
Patient's supernatant serum (serum after cryoprecipitation)	6.83	3.21	0.32	0.91	1.13	....	1.26
Patient's serum after cortisone therapy	6.89	4.05	0.22	0.68	1.08	....	0.86
Cryoprotein in saline	....	....	....	....	....	....	0.30*

* Cryoprotein was determined from a concentrated solution; therefore, this quantity does not reflect the concentration in the patient's serum.

was determined by the method of Weichselbaum.[24] The result was equated with the number of square units under the corresponding electrophoretic curve. One unit of area corresponded to 0.027 gm. per 100 ml. of solution. This standard was used in all the electrophoretic calculations in this investigation.

The principal error in this method of estimating protein concentrations lies in the assumption that the intensity of the dye bound by the protein in the electrophoretic strips is directly proportional to the protein concentration. In general, the albumin binds a greater proportion of dye than does the globulin.[10]

Spectrophotometry. The ultraviolet absorption spectrum of the cryoprecipitate in 0.9 per cent saline solution was determined. The apparatus was a Beckman quartz spectrophotometer with a hydrogen discharge tube operated at room temperature (27°C.).

RESULTS

Serum Electrophoretic Patterns. The patterns of the protein distribution in the serum samples taken from the patient described in this study are, in each case, different from that of the pooled normal serum. (Table I and Figs. 1 to 4.) They are also different from each other. The greatest alteration in the electrophoretic pattern is found in the "T" component with a mobility between the beta and gamma globulins. This is not present in the normal serum but it is prominent in the patient's whole serum. (Fig. 2.) After separation of the cold-precipitable fraction this element disappears and the electrophoretic curve has a greater resemblance to normal. This segment, "T," is similarly absent in the sample taken in the period after cortisone treatment.

The gamma segment in this patient is difficult to evaluate because of its proximity to the "T" globulin. For this reason the two fractions are listed jointly in Table I. Although there is a decrease of these peaks in the whole serum after separation of the cryoprotein, the combined "T" and gamma segments remain in greater quantity in the supernatant than in the normal. After cortisone administration the gamma globulin is of lesser magnitude than that in the pooled serum.

Other variations are also apparent. The beta globulin is relatively constant in each sample of the patient's serum but the values are all higher than those of the normal. The alpha-2 component, on the other hand, is of the same order of magnitude in the normal, supernatant and whole serum, but lower in the serum after cortisone administration. The alpha-1 globulins are nearly equal in the normal samples and those after cortisone therapy, and higher in the whole and supernatant serums. There is a decreased amount of albumin in the serum from which cryoprotein has been separated. This quantity is smaller than that in the serum after cortisone has been administered and the normal pooled serum. The variations in the individual fractions and in the total protein concentrations are illustrated in Table I.

Electrophoresis of Cryoprotein. Figure 5 illustrates the electrophoretic curve inscribed by the

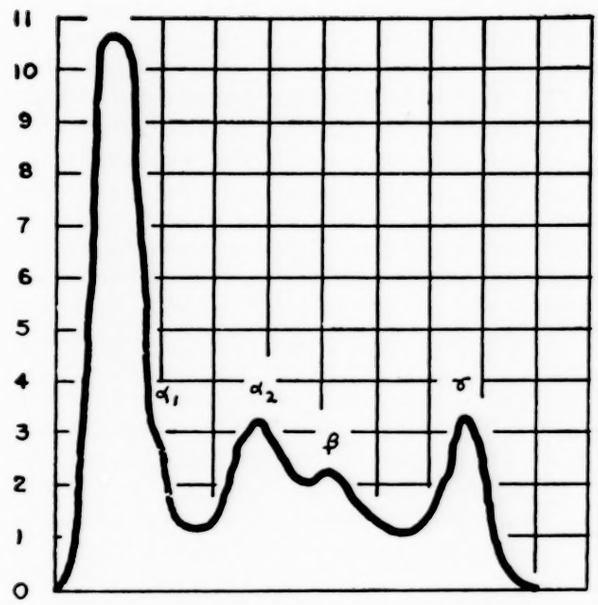

FIG. 1. Pooled normal human serum.

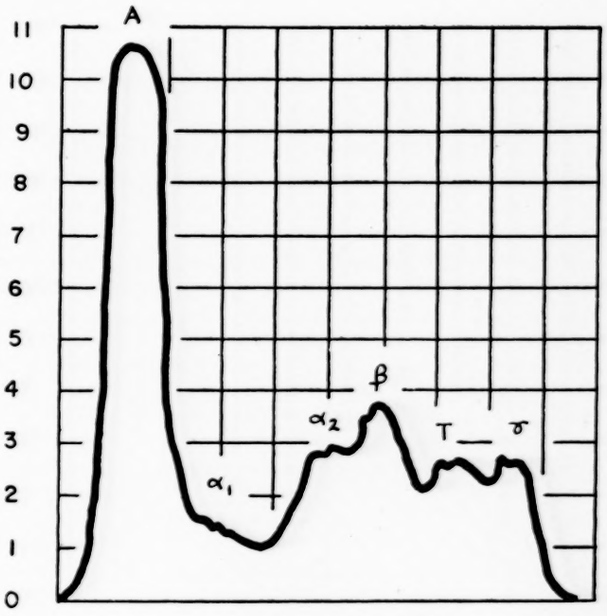

FIG. 2. Patient's whole serum.

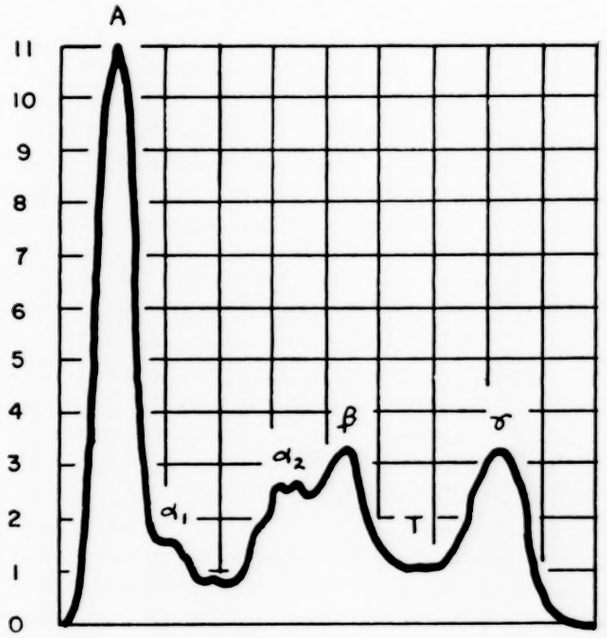

FIG. 3. Patient's supernatant serum (after cryoprecipitation).

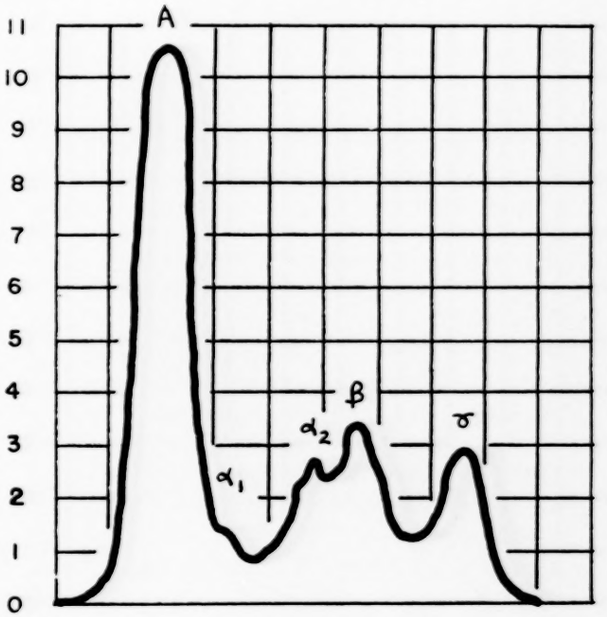

FIG. 4. Patient's serum after administration of cortisone.

scanner when the "purified" cold-precipitable protein was analyzed. There is only one peak, and the slopes of both sides are of approximately the same pitch. The mobility is approximately that of the "T" component seen in the whole serum. (Fig. 5.) The solution represents a concentrate of the purified materials from several serum samples. Therefore, the quantity shown in Table 1 is not indicative of the amount precipitated per unit volume of whole serum.

Other Properties of the Cryoprecipitate. The precipitate obtained after incubation of the serum at 5°C. was soluble in water, 0.9 per cent saline solution, 0.01 N HCl and 0.01 N NaOH. However, it was cold-precipitable only from the normal saline solution. Heating at over 40°C. for a

few minutes resulted in coagulation and permanent insolubility in water and saline solution. The ultraviolet absorption spectrum indicated a maximum opacity at 2700 A in 0.9 per cent saline solution. Further studies are planned after the effects of cortisone treatment disappear.

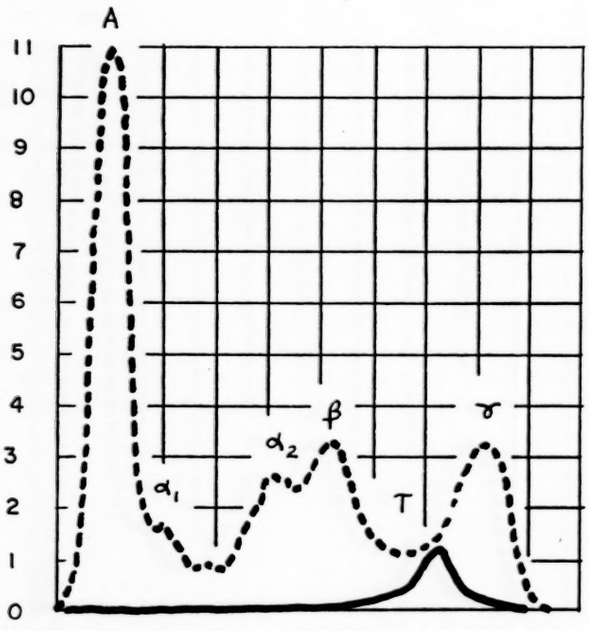

FIG. 5. Cryoprotein in saline solution (dotted line indicates supernatant serum).

Refrigeration of Serum after Administration of Cortisone. The serum obtained from the patient after treatment for seven days with oral cortisone, was placed in the refrigerator at 5°C. After two weeks' incubation no precipitate could be seen after centrifugation for fifteen minutes at 30,000 r.p.m. at 0°C. It was concluded that no cryoprecipitate was present in significant quantities in the sample of serum after cortisone therapy.

COMMENTS

The electrophoretic pattern of the "purified" saline solution of the cold-precipitable protein was that of a single unit represented by a symmetrically shaped curve. This would seem to indicate the presence of a homogeneous (but not necessarily homomolecular) protein with a mobility between the beta and gamma globulins. However, it would be possible for another component, having the same mobility at pH 8.6 and under the conditions of the experiment, to remain undetected. In addition, one or more proteins not exceeding 1 or 2 per cent of the total protein concentration would not be observed. Finally, the process of "purification" might have eliminated cold-precipitable proteins as well as normal protein fractions precipitated by adsorption with the cryo component.

The main alteration in the supernatant relative to the whole serum was the disappearance of an abnormal "T" component with mobility between the beta and gamma globulins. The alpha-1, alpha-2 and beta components were insignificantly changed. The albumin, however, was decreased. It is probable that this change was due to precipitation by adsorption on the "T" component. This follows from the fact that the albumin fraction showed no tendency to precipitate in the cold from the serum after cortisone was administered although its concentration was approximately as great as in the whole serum.

Comparison of the electrophoretic curves identified cryoprotein with the "T" component. The alpha-1, alpha-2 and beta components were not affected by cryoprecipitation as indicated by a comparison between the patterns of the whole and supernatant serums. (Figs. 1 and 2.) The albumin was decreased in the supernatant serum but was present in the sample after cortisone therapy in concentrations almost as great as in the whole serum. The latter, after two weeks' incubation at 5°C., showed no tendency to yield a precipitate. It would therefore appear that the cryoprotein in this case is a substance with mobility between the beta and gamma globulins.

The serum after cortisone administration contained no "T" fraction and showed a significant decrease in three of its globulin components: the alpha-1, alpha-2 and gamma globulins. The beta globulin fraction seemed to be unchanged. (Table I.) Further study is indicated on the significance of this occurrence. It has been shown by several investigators that ACTH and cortisone affect the serum protein concentrations.[18] However, investigations in this field are conflicting, particularly in regard to the effect on antibodies. Dougherty and co-workers[5] claimed that the dissolution of lymphoid tissue by adrenocortical steroids or ACTH in experimental animals is accompanied by a rise in the antibody titer and the concentration of gamma globulin in the blood. However, controlled experiments in rabbits immunized with a polyvalent pneumococcal vaccine have revealed definitely impaired antibody formation in animals receiving ACTH or cortisone.[2,6] Comparable experiments in man have failed to reveal any decrease in antibody

production.[14,19] The changes of the serum proteins of the patient in this study after treatment with cortisone are therefore interesting in view of the association of cryoprotein, migrating in the "T" globulin component, with urticaria.

Proteins with similar electrophoretic mobilities may differ in other respects. This is particularly true in relation to different animal species. It may be significant, however, that in connection with horse antipneumococcal serum, a fraction with mobility between the beta and gamma positions was first reported by Tiselius and Kabat in 1939.[21] It was designated the "T" component[9] and it disappeared on removal with specific polysaccharide. This finding was confirmed by Smetana and Shemin[16] and by Van der Scheer et al.[23] In man, globulins with such intermediate mobility have sometimes been designated "M" globulins. They are frequently encountered in the serums of individuals with multiple myeloma.

Human Wassermann antibody has been found to have a mobility in the "T" component,[4] while antibody activity of bovine plasma and colostrum was associated with two components. One migrated between the beta and gamma globulins and the other one with the gamma globulin.[17] On the other hand, the electrophoretic patterns of all hyperimmune rabbit serums showed antibody to be associated with the gamma globulin fraction.[9]

Van der Scheer[22] has separated horse antiserums experimentally into three distinct classes: (1) those in which the gamma globulin is increased, (2) those in which a new electrophoretic component "T" is present along with the normal gamma globulin (e.g., tetanus antitoxin), and (3) those in which the "T" component is present along with an enhanced gamma globulin.

A somewhat similar pattern seems to occur in human subjects with multiple myeloma. With respect to the electrophoretic mobilities, Gutman[7b] observed that most serums in multiple myeloma fall into one of three tentative classifications: (1) serums with protein increments composed chiefly of gamma globulins or abnormal components migrating with the mobility of gamma globulins, (2) serums with protein increments giving a variety of anomalous electrophoretic patterns, usually showing extra components migrating with the mobility of beta globulins or intermediate between the beta and gamma globulins, and (3) serums with apparently normal electrophoretic patterns. Osserman and Lawler[15a] described abnormal serum and urine proteins with mobilities in the gamma, beta, alpha-2 and beta components in thirty-five cases of this disease.

The possibility that some of the abnormal proteins in multiple myeloma represent antibodies has been suggested.[7b] Slater et al.[15b] have recently investigated the immunologic relationship among myeloma proteins. They found that every one of the myeloma proteins studied was immunologically different, indicating individual specificity. Although definite evidence was lacking, the authors found it tempting to consider the myeloma proteins as intermediates in the synthesis of gamma globulins and antibodies, the more closely related myeloma proteins having most of the determinant groups of gamma globulin.

The mobility of abnormal proteins in cases with cryoproteinemia also vary. Barr et al.[1] reported two cases with an "M" component in the serum electrophoretic pattern. Osserman and Lawlor[15a] identified cryoprotein in the gamma globulins. The subject in this study did not have multiple myeloma; the serum cryoprotein was found principally in the "T" component, although some may have had the characteristics of gamma globulin. Further investigation in similar cases may show a distribution in the other globulin components, as in multiple myeloma.

The similarity of cryoprotein to immune bodies is not limited to the electrophoretic mobility. The molecular weight,[12,3b] 190,000 to 200,000, is very near that of 195,000 determined for human pneumococcal antibody.[8] Furthermore, Svartz and Schlossman[20] have recently reported the presence of a hemagglutinating factor in the cold precipitate of 95 per cent of 100 patients with rheumatoid arthritis. Finally, the subject in this study reacted to cold with urticaria and pruritus. When these symptoms could be elicited by exposure to cold, cryoprotein could always be extracted from the serum. After treatment with cortisone the symptoms disappeared and serum cryoprecipitation no longer occurred. These data suggest that cryoproteins, like the abnormal fractions in multiple myeloma, represent immune bodies. Perhaps further investigations will clarify this important problem.

SUMMARY

1. The electrophoretic distribution of the serum proteins in cryoproteinemia prior to and

following separation of the cryoprecipitate, and after the administration of cortisone, is analyzed and compared to normal pooled human serum.

2. The electrophoretic curve of "purified" cryoprotein in saline solution is illustrated. Cryoprotein, in the case described in this study, is represented electrophoretically by a "T" component which migrates between the beta and gamma globulins.

3. This component was absent after treatment with cortisone for six days. Concomitantly, no cryoprotein was obtained from the serum and the patient no longer reacted abnormally to cold.

4. The similarities between cryoprotein and immune bodies are discussed. This study seems to illustrate the suppressive effects of an adrenocortical hormone on serum globulins and on a "T" component with immune body properties.

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REFERENCES

1. Barr, D. P., Reader, G. G. and Wheeler, C. H. Cryoglobulinemia. I. Report of two cases with discussion of clinical manifestations, incidence, and significance. *Ann. Int. Med.*, 32: 6, 1950.
2. Bjørneboe, M., Fischel, E. E. and Stoerk, H. C. The effect of cortisone and adrenocorticotrophic hormone on the concentration of circulating antibody. *J. Exper. Med.*, 93: 37, 1951.
3. (*a*) Block, R. J., Durrum, E. L. and Zweig, G. A Manual of Paper Chromatography. New York, 1955. Academic Press, Inc.; (*b*) von Bornsdorff, B., Groth, H. and Packalen, T. On the presence of a high-molecular crystallizable protein in blood serum in myeloma. *Folia haemat.*, 59: 184, 1938.
4. Davis, B. D., Moore, D. H., Kabat, E. A. and Harris, A. Electrophoretic, ultracentrifugal and immunochemical studies on Wassermann antibody. *J. Immunol.*, 50: 1, 1945.
5. Dougherty, T. F., White, A. and Chase, J. H. Relationship of effects of adrenocortical secretions on lymphoid tissue and on antibody titer. *Proc. Soc. Exper. Biol. & Med.*, 56: 28, 1944.
6. Germuth, F. G. and Ottinger, B. Effect of 17-hydroxy-11-dehydrocorticosterone (compound E) and ACTH on Arthus reaction and antibody formation in the rabbit. *Proc. Soc. Exper. Biol. & Med.*, 74: 815, 1950.
7. (*a*) Holmberg, C. G. and Grönwall, A. Ein neues krystallinisches Serumglobulin. *Ztsch. f. physiol. Chem.*, 273: 199, 1942; (*b*) Gutman, A. B. The plasma proteins in disease. Advances in Protein Chemistry, vol. 4, p. 156. Edited by Anson, M. L. and Edsall, J. T. New York, 1948. Academic Press.
8. Kabat, E. A. The molecular weight of antibodies. *J. Exper. Med.*, 69: 103, 1939.
9. Kabat, E. A. and Mayer, M. M. Experimental Immunochemistry. Springfield, Ill., 1948. Charles C Thomas.
10. Köiw, E., Wallenius, G. and Grönwall, A. Paper electrophoresis in clinical chemistry. *Scandinavian J. Clin. & Lab. Investigation.*, 4: 47, 1952.
11. Lerner, A. B., Barnum, C. P. and Watson, C. J. Studies of cryoglobulins. II. The spontaneous precipitation of protein from serum at 5 degrees centigrade in various disease states. *Am. J. M. Sc.*, 214: 416, 1947.
12. Lerner, A. B. and Greenberg, G. R. A homomolecular serum protein with anomalous solubilities. *J. Biol. Chem.*, 162: 429, 1946.
13. Lerner, A. B. and Watson, C. J. Studies of cryoglobulins. I. Unusual purpura associated with the presence of a high concentration of cryoglobulin. *Am. J. M. Sc.*, 214: 410, 1947.
14. Mirrick, G. S. The effects of ACTH and cortisone on antibodies in human beings. *Bull. Johns Hopkins Hosp.*, 88: 332, 1951.
15. (*a*) Osserman, E. F. and Lawlor, D. P. Abnormal serum and urine proteins in thirty-five cases of multiple myeloma as studied by filter paper electrophoresis. *Am. J. Med.*, 18: 462, 1955; (*b*) Slater, R. J., Ward, S. M. and Kunkel, H. G. Immunological relationships among myeloma proteins. *J. Exper. Med.*, 101: 85, 1955.
16. Smetana, H. and Shemin, D. Studies on photooxidation of antigen and antibodies. *J. Exper. Med.*, 73: 223, 1941.
17. Smith, E. L. Immune proteins of bovine colostrum and plasma. *J. Biol. Chem.*, 164: 345, 1946.
18. Sprague, R. G. Cortisone and ACTH. *Am. J. Med.*, 10: 567, 1951.
19. Svartz, N. The effect of ACTH on the agglutination with sensitized red sheep cells in rheumatoid arthritis. *Acta med. Scandinav.* (suppl. 246), p. 240, 1950.
20. Svartz, N. and Schlossman, K. A serum cold precipitable hemagglutinating factor in rheumatoid arthritis. *Acta med. Scandinav.*, 149: 83, 1955.
21. Tiselius, A. and Kabat, E. A. Electrophoresis of immune serum. *J. Exper. Med.*, 69: 119, 1939.
22. Van der Scheer, J., Wykoff, R. W. G. and Clarker, F. H. The electrophoretic analysis of several hyperimmune horse sera. *J. Immunol.*, 39: 65, 1940.
23. Van der Scheer, J., Lagsdin, J. B. and Wykoff, R. W. G. Electrophoretic and ultracentrifugal analyses of antipneumococcal horse sera. *J. Immunol.*, 41: 209, 1941.
24. Weichselbaum, T. E. An accurate and rapid method for the determination of protein in small amounts of blood, serum and plasma. *Am. J. Clin. Path., Tech. Sect.*, 10: 40, 1946.
25. Wintrobe, M. M. and Buell, M. V. Hyperproteinemia associated with multiple myeloma. *Bull. Johns Hopkins Hosp.*, 52: 156, 1933.
26. Wirtschafter, Z. T., Williams, D. W. and Gaulden, E. C. Unpublished data.

Studies on a Patient with Severe Hyperinsulinism Treated with Cortisone for Three Years*

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SEVERE hyperinsulinism is not an uncommon entity. In a report by Howard et al.,[1] 400 cases due to hyperfunctioning tumors of the pancreas were reviewed. In recent years cortisone and adrenocorticotropin (ACTH) have been reported to have afforded temporary relief of hypoglycemia in several cases.[2–5] We are presenting studies on a patient who has been treated with cortisone† for three years.

METHODS AND PROCEDURES

All blood glucose analyses were carried out on venous heparinized whole blood that was immediately precipitated at the bedside to avoid glycolysis. Glucose was determined by the Nelson-Somogyi technic,[6] plasma potassium in a flame photometer, and eosinophils by the technic of Randolph.[7]

CASE REPORT

E. J., a sixty-one year old, white male engineer, was admitted to the Psychiatric Division of Bellevue Hospital on September 28, 1951, because of violent, uncontrollable behavior. While at work he had suddenly become agitated and incoherent. On arrival at the hospital he was quiet and after receiving food became entirely lucid and rational.

He had been in good health until August, 1950, when he had his first attack. At that time he had worked on the night shift, eaten, and had gone to sleep at 8 A.M. At 1 P.M. he awoke with a pounding heart, shaking, confused and feeling that something was wrong with his mind. Two similar episodes occurred, one in January, 1951, six hours after eating, and one in March, 1951, which had also awakened him from sleep. In the past year he had noticed that he became confused, nervous and sweaty in the morning if he did not eat shortly after awakening. On the day of admission these symptoms began about noon, while working, and he remembered nothing thereafter until his admission to the hospital.

Past history was significant only in the following facts: Twenty years ago he drank heavily "on weekends" but in the past several years alcohol was limited to occasional beers. He had had frequent upper respiratory infections and one bout of pneumonia which required hospitalization.

On physical examination he was a well developed, muscular man, 5 feet 7 inches in height, weighing 155 lb., and looking younger than his stated age. Temperature was 98.6°F., pulse rate 96 per minute, respiratory rate 20 per minute. There were a few crepitant rales at the base of the right lung. Cardiac findings were normal except for a soft systolic murmur heard over the apex. The blood pressure was 180/100 and varied subsequently from 116–180/70–88. The right lobe of the liver was palpable two fingerbreadths below the costal margin. Neurologic examination was normal.

Urinalysis also was within normal limits. The hemoglobin was 13 gm. per cent, red blood cells 4.8 million, white blood cells 6,450 with 50 per cent polymorphonuclear cells, 30 per cent lymphocytes, 14 per cent monocytes, 2 per cent eosinophils, 1 per cent basophils and 3 per cent immature forms. On admission the fasting blood sugar (FBS) was 32 mg. per cent, and on subsequent days varied from 15 to 35 mg. per cent. Skull, abdominal and gastrointestinal x-rays were normal. The feces were guaiac-negative, and trypsin was present in normal amounts. Serum total protein was 5.0 gm. per cent, albumin 2.1 gm. per cent, globulin 2.9 gm. per cent, thymol turbidity 11 units, serum sodium 137 mEq./L., inorganic phosphate 2.8 mg. per cent, plasma CO_2 41 vol. per cent.

The patient was given a high protein, low fat diet, and orange juice was kept at his bedside. He would usually arise at about 4 A.M. with nervousness and hunger which were relieved after drinking the orange

† We are indebted to Merck & Co., Rahway, New Jersey, for the cortisone used in the treatment of this patient.

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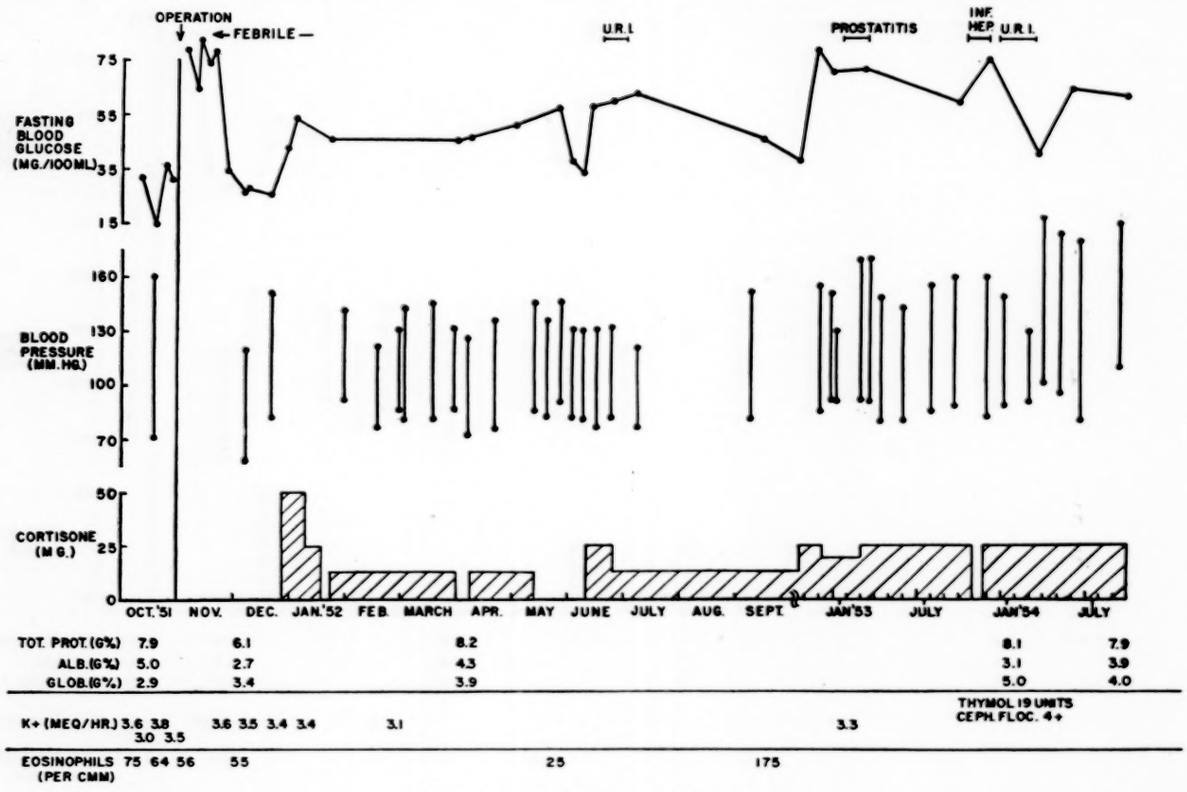

FIG. 1. Summary of patient's course.

juice. A summary of the patient's course is shown in Figure 1.

An epinephrine test performed on the morning of October 11th produced a 65 per cent fall in eosinophils but no rise in blood glucose. (Table I.) Steroid excretion measured on October 20th was 9.5 mg. ketosteroid and 1.4 mg. corticoids in twenty-four hours. A glucose tolerance test was performed on October 18th. (Table II, Fig. 2.) On that morning the patient was extremely nervous and hungry. He had palpitations, his skin was pale and cool, and his breathing deep and rapid. Blood pressure was 160/70, heart rate 72 per minute, and blood glucose 15 mg. per cent. Three minutes after drinking glucose in water the patient felt better, the blood pressure fell to 120/60, the heart rate to 64 per minute, and the skin became warmer with more normal color. Four hours later the blood pressure and heart rate had risen again and the blood glucose had fallen to 29 mg. per cent. The blood eosinophils did not change significantly in four hours (64 to 48/cm.). The plasma potassium rose and remained elevated for the four hours.

Electrocardiographic tracings were taken on three occasions. These showed variations in T-3 which did not appear to be related to the blood levels of glucose or potassium. On October 18th, when the glucose level was 63 mg. per cent and the potassium was 3.9 mEq./L., an electrocardiogram showed an inverted T-3. On October 23rd when the symptoms of hypoglycemia occurred the blood glucose was 36 mg. per cent, the potassium 3.8 mEq./L. and T-3 was upright. At this time symptoms were relieved by breakfast. The blood glucose rose to 43 mg. per cent and the plasma potassium was 3.6 mEq./L.; in the electrocardiographic tracing T-3 had become inverted.

TABLE I
EFFECT OF INJECTION OF EPINEPHRINE AND INSULIN*

Time (min.)	Blood Glucose (mg./100 ml.)	Plasma Potassium (mEq./L.)	Eosinophils (per cu. mm.)	Heart Rate (per min.)	Blood Pressure
Epinephrine,† 0.3 mg. subcutaneously (October 11, 1951)					
0	32	3.6	75	64	.....
15	33	4.0	88	72	.....
30	28	3.9	..	66	.....
240‡	98	4.0	26	68	.....
Insulin,† 2 units intravenously (December 21, 1951)					
0	26	3.4	..	72	150/80
5	..	...	..	72	165/70
10	25	3.1	..	72	145/80

* Studies were begun before breakfast, although patient had orange juice at 2 and 4 A.M.
† The epinephrine or insulin was injected at 0 minutes.
‡ Patient ate breakfast 90 minutes after injection of epinephrine.

TABLE II

RESULTS OF ORAL GLUCOSE TOLERANCE TESTS (50 GM.)

Date	Conditions	Time (min.)	Blood Glucose (mg./100 ml.)	Plasma Potassium (mEq./L.)	Eosinophils (per cu. mm.)	Blood Pressure	Heart Rate (per min.)
10/18/51	Preoperative	0	15	3.0	64	160/70	72
		30	57	3.6	48	110/60	64
		60	73	3.5	...	120/60	62
		120	63	3.9	...	110/60	54
		240	29	4.2	48	140/70	64
11/16/51	16 days postoperatively	0	74	...	...	.....	..
		30	122	...	...	.....	..
		60	129	...	...	.....	..
		120	148	...	...	.....	..
		180	138	...	...	.....	..
12/7/51	37 days postoperatively; draining abdominal sinus; temperature 101°F.	0	28	3.5	55	120/55	74
		30	49	3.8	39	98/48	68
		60	63	3.6	...	94/50	66
		120	89	3.7	...	94/50	60
		180	77	3.9	...	94/50	66
		240	49	3.8	53	106/58	66
1/4/52	Received 50 mg. cortisone daily for 7 days	0	53	3.4	...	154/80	60
		30	101	3.7	...	144/72	60
		60	92	3.7	...	150/74	60
		120	101	4.2	...	140/75	60
		180	79	3.7	...	150/80	54
		240	61	3.9	...	162/82	60
1/23/52	No cortisone for 5 days	0	45	...	...	136/74	80
		30	108	...	...	116/68	69
		60	111	...	...	104/64	69
		120	114	...	...	112/68	60
		180	80	...	...	126/72	60
		240	54	...	...	130/70	66
3/31/52	Received 12.5 mg. cortisone daily for 68 days	0	45	3.1	...	130/85	64
		30	83	3.8	...	130/90	66
		60	101	...	...	140/90	58
		120	91	3.9	...	.....	..
		210	51	3.8	...	150/85	60
		240	51	4.0	...	160/90	64
9/16/52	Receiving 12.5 mg. cortisone daily	0	46	...	175	.....	..
		30	77	...	...	.....	..
		60	78	...	...	.....	..
		120	102	...	...	.....	..
		180	54	...	...	.....	..
		240	48	...	148	.....	..

The patient continued to have hypoglycemic attacks on awakening and the FBS was consistently below 35 mg. per cent. On November 1st an exploratory laparotomy was performed. The pancreas was found to be of normal size and felt moderately fibrosed. A small, 1.0 by 0.75 cm., spherical mass of firmer consistency than the rest of the pancreatic tissue was excised but no tumor was found. A lymph node and the gallbladder were removed, a liver biopsy done and a pancreatogram made. The pathologic report revealed the islets of Langerhans to be of moderate size with the exception of one such structure which was about twice the size of the others seen in the section; the ducts and vessels were not unusual.

Microscopic sections of the liver showed infiltrates of lymphocytes and proliferating bile ducts in the portal areas, and a mild increase in fibrous tissue.

The postoperative course was complicated by the development of a fistulous tract following removal of the T-tube in the common bile duct. Jaundice

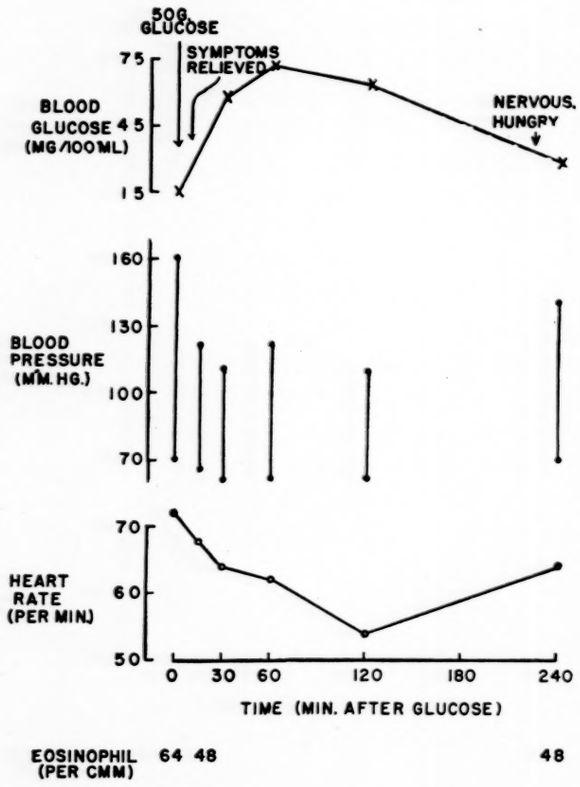

FIG. 2. Effects of glucose on symptoms, blood pressure, heart rate and blood sugar.

developed, and for about two months the patient ran a fever of 101 to 102°F. There were no attacks of hypoglycemia for the first four weeks; the FBS ranged from 65 to 80 mg. per cent. (Fig. 1.) A glucose tolerance test on November 16th (Table II) showed a normal response; in fact, the three-hour blood glucose level was elevated. On November 28th, however, nervousness and hunger reappeared in the morning, and the blood glucose was found to be 35 mg. per cent. The patient was given frequent feedings of protein foods and supplementary feedings of orange juice. Another glucose tolerance test was done on December 7, 1951. (Table II.) Symptoms of hypoglycemia again responded to glucose within two minutes; the heart rate fell rapidly; the blood pressure which had remained comparatively low throughout the patient's febrile postoperative period also fell within minutes from 120/55 to 98/48.

An effort was made to test the patient's sensitivity to insulin. On the morning of December 21st, two units of insulin were injected intravenously. (Table I.) The initial blood sugar level was 26 mg. per cent. Five minutes after the injection the patient began to grimace and cry, and turned from side to side. The blood glucose taken at ten minutes showed no change from the initial value, although the plasma potassium had fallen 0.3 mEq./L. The reaction was readily controlled with intravenous glucose.

As the patient continued to suffer from frequent hypoglycemic attacks, treatment with 50 mg. of cortisone daily was begun on December 28th. This was given in divided doses before each meal and at bedtime. Within five days the FBS had risen to 42 mg. per cent and two days later to 52 mg. per cent. The symptoms of hypoglycemia disappeared. During a glucose tolerance test performed on January 4, 1952, the third and fourth hour blood glucose levels were maintained well above symptomatic levels. (Table II.) The dose of cortisone was gradually reduced and on January 23, 1952, the patient was discharged to the Clinic on a regimen of 12.5 mg. daily. At this time the FBS was 45 mg. per cent. Physical examination at this time showed the liver to be enlarged six fingerbreadths below the costal margin. The enlarged liver persisted until March, 1953. He also had a draining incisional sinus which persisted for about six more months.

Several times in the following months the cortisone was discontinued for short periods. On April 7th, after cortisone had been stopped for one week, the FBS remained unchanged at 46 mg. per cent. On May 12th the cortisone was again stopped, two weeks later the FBS was 57 mg. per cent but then fell to 38 mg. per cent on June 2nd, and 34 mg. per cent on June 9th, with recurrence of symptoms. One week after resumption of 25 mg. of cortisone daily, the FBS rose to 58 mg. per cent, and two weeks later to 60 mg. per cent. While receiving 12.5 mg. cortisone daily, the FBS fell to 46 mg. per cent on September 16th, and to 38 mg. per cent on October 20th. The dose of cortisone was increased to 25 mg. daily and the FBS rose to 78 mg. per cent on November 24th. The patient was hospitalized at another hospital for infectious hepatitis during October and November, 1953. During this time cortisone was discontinued for one week and the blood glucose values were reported as falling to 40 and 45 mg. per cent. Except for this interval, the patient has been maintained on 25 mg. daily. The FBS, except on one occasion, has been above 60 mg. per cent, and no hypoglycemic attacks have occurred. The blood pressure, which early in the disease showed extreme lability, has now reached a consistently hypertensive level of about 190/110. His liver is now palpable two fingerbreadths below the costal margin and liver function tests show some impairment of function. The serum albumin level is 3.1 gm. per cent, globulin 5.0 gm. per cent, cephalin flocculation 4 plus and thymol turbidity 20 units.

COMMENTS

The severity of the hypoglycemia and the course of the disease make it probable that the patient is suffering from "organic" hyperinsulinism. The most permanent relief would be expected from resection of the pancreas but he has refused another operation. In this patient cortisone has proved satisfactory for a remarkably long period of time in maintaining the blood glucose levels and in preventing the symptoms of hypoglycemia. In cases reported by other observers cortisone has been used for only short periods, and de Peyster and Gilchrist[5] found it ineffective in relieving symptoms of hypoglycemia in their patient. In the case we studied the augmented blood glucose level persisted for two to three weeks after cortisone was discontinued, whereas Brown et al.[2] reported hypoglycemic levels of blood glucose in a patient one day after cortisone was discontinued. That patient had received 200 mg. of cortisone daily.

The cardiovascular reaction observed in the patient we studied resembles that induced in the cat by Cannon et al.[8] following the injection of insulin. These investigators found that when the blood glucose fell to 70 mg. per cent (using the Folin-Wu method which gives values 15 to 20 mg. per cent higher than the Nelson-Somogyi technic), the heart rate suddenly increased. Following the injection of glucose the heart rate fell rapidly. The increased heart rate was ascribed to adrenal medullary secretion since it did not occur if the splanchnic nerves to the medulla were cut. On at least four occasions the patient we studied showed a similar reaction to his own insulin secretion and to the ingestion of glucose. In addition, changes in blood pressure were observed. On October 18 and December 7, 1951, and on January 23, 1952 (Table II), the ingestion of glucose was followed by an immediate decrease in blood pressure and heart rate. Three to four hours later, when the level of blood glucose was falling, the blood pressure and heart rate began to increase. Similar changes in blood pressure and heart rate were observed on October 18th when the blood glucose level was below 60 mg. per cent; on December 7th when the glucose level was 49 mg. per cent, and on January 23rd when the glucose levels were between 80 and 54 mg. per cent. On March 31, 1952, the blood pressure did not change following the ingestion of glucose; in fact, it rose as did the heart rate even though the blood glucose later was only 51 mg. per cent.

The vasomotor changes occurred at glucose levels well above those at which the hypoglycemic symptoms appeared. (Table III.) In the cat an increase in heart rate was also observed to occur before any other evidence of sympathetic activity, such as changes in the pupils or nictitating membrane, was observed. The vasomotor changes can be related to the blood glucose changes by supposing that the two hormones of the adrenal medulla, nor-epinephrine and epinephrine, are released at different blood glucose levels. The initial response to a falling blood glucose level could be a release of nor-epinephrine which acts predominantly by increasing blood pressure and has little effect on symptoms in humans, or on the eyes of the cat.[9] As the blood glucose level continues to fall, epinephrine is secreted, accelerating glycogenolysis, increasing the output of ACTH and inducing tachycardia, palpitation, anxiety and pallor. The recent demonstration of Hillarp and Hökfelt[10] of specific pigment cells in the adrenal medulla of the rat and of the cat, which selectively secrete epinephrine and nor-epinephrine, would lend support to this theory. Sustained hypoglycemia was shown to deplete the adrenal medulla of epinephrine without change in nor-epinephrine content.[11] It is quite possible, under the conditions of those experi-

TABLE III
RELATIONSHIP OF SYMPTOMS TO BLOOD GLUCOSE AND PLASMA POTASSIUM LEVELS

Symptoms	Blood Glucose (mg. %)	Plasma Potassium (mEq./L.)	Conditions
Anxiety, palpitation, hyperpnea	15	3.0	Fasting
Disoriented, grimacing	25	3.1	10 min. after insulin injection
Hungry, anxious	26	3.3	Fasting
Nervous	29	4.2	4 hr. after glucose
Nervous	29	3.6	Fasting
Nervous	29	3.5	Fasting
Hungry	32	3.6	Fasting
Weak	34	...	Fasting
Nervous, hungry	36	3.8	Fasting
None	38	...	Fasting
None	38	4.2	4 hr. after glucose
None	38	...	Fasting during cortisone therapy
None	45	3.1	Fasting during cortisone therapy

ments, that an early transient secretion of nor-epinephrine would have been undetected.

As the blood glucose level in this patient fell below 38 to 36 mg. per cent symptoms of hypoglycemia appeared. (Table III.) This level is identical with that at which the pituitary-adrenocortical system is activated in the rat[12] and in man.[13] Since epinephrine has been shown to be the probable mediator of this activation,[14] whereas nor-epinephrine has no action on the pituitary,[15] it would appear that the predominant secretion is epinephrine. It may well be that mild hyperinsulinism is involved in individuals with labile hypertension, particularly in those patients who exhibit blood pressure and heart rate changes in relation to food intake.

It has been noted for many years[16] that hypoglycemia will produce a wide variety of cardiovascular symptoms. Data on the relation of the severity of the symptoms to the absolute level of blood glucose have been difficult to obtain from recorded cases because of the different blood sugar methods used by various investigators and because of the timing of the blood specimens in relation to the severity of the symptoms. It is frequently stated that blood sugar levels below 35 mg. per cent are accompanied by hypoglycemic symptoms. However, this level is not invariably a critical one, as illustrated by Lukens et al.[17] who observed no hypoglycemic symptoms in a patient with a blood sugar level of 15 mg. per cent.

The fasting plasma potassium varied with the glucose level (Table III) but this seems to have played, at most, a secondary role in the development of symptoms. The plasma potassium increased after the ingestion of glucose and remained high even when the glucose level fell and the symptoms reappeared. For example, on one occasion hypoglycemic symptoms developed with a blood glucose of 29 mg. per cent. The plasma potassium level remained normal (4.2 mEq./L.). On another occasion, while receiving cortisone, there were no symptoms when the FBS was 45 mg. per cent at which time the potassium was only 3.1 mEq./L.

It should be mentioned that this patient presented no evidence of abnormal adrenocortical activity. The ketosteroid and corticoid excretions were normal, the blood eosinophil level was within normal limits and showed a normal response to epinephrine. In this respect this patient is similar to the one reported by Brown et al.[2]

SUMMARY

Studies are presented in a patient with severe hypoglycemia which has been controlled for three years by the administration of 25 mg. of cortisone daily.

The patient exhibited findings which emphasize the relationship between vasomotor lability and hypoglycemia. As his blood glucose level fell below 70 to 60 mg. per cent the blood pressure and heart rate rose. When glucose was ingested, the blood pressure and heart rate fell immediately. A further decrease in blood glucose to a level of 38 to 36 mg. per cent produced hypoglycemic symptoms.

It is suggested that nor-epinephrine and epinephrine are released from the human adrenal medulla in response to different levels of hypoglycemia. It is also suggested that mild hyperinsulinism may be suspected in an individual who shows such cardiovascular changes in relation to food intake.

REFERENCES

1. Howard, J. M., Moss, N. H. and Rhoads, J. E. Collective review: hyperinsulinism and islet cell tumors of the pancreas with 398 recorded tumors. *Internat. Abst. Surg.*, 90: 417, 1950, in *Surg., Gynec. & Obst.*, 1950.
2. Brown, H., Hargreaves, H. P. and Tyler, F. H. Islet-cell adenoma of the pancreas. Metabolic studies on a patient treated with corticotropin and cortisone. *A. M. A., Arch. Int. Med.*, 89: 951, 1952.
3. McQuarrie, I., Ziegler, M. R., Wright, W. S., Bauer, E. G. and Ulstrom, R. A. Further studies on the effects of ACTH on spontaneous hypoglycemosis, in Proc. of Second Clin. ACTH Conf., Therapeutics, vol. 2., p. 69. Philadelphia, 1951. The Blakiston Co.
4. Brown, G. M. The effect of ACTH in a case of beta islet cell adenoma of the pancreas. *Am. J. Digest. Dis.*, 18: 145, 1951.
5. de Peyster, F. A. and Gilchrist, R. K. Clinical response of spontaneous hypoglycemia to dietary and drug therapy. *J. A. M. A.*, 155: 884, 1954.
6. Nelson, N. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.*, 153: 375, 1944.
7. Randolph, T. G. Blood studies in allergy. I. The direct counting chamber determination of eosinophiles by propylene glycol aqueous stains. *J. Allergy*, 15: 89, 1944.
8. Cannon, W. B., McIver, M. A. and Bliss, S. W. Studies on the conditions of activity in endocrine glands. XIII. A sympathetic and adrenal mechanism for mobilizing sugar in hypoglycemia. *Am. J. Physiol.*, 69: 46, 1924.
9. Goldenberg, M. Adrenal medullary function. *Am. J. Med.*, 10: 627, 1951.
10. Hillarp, N. and Hökfelt, B. Cytological demon-

stration of noradrenaline in the suprarenal medulla under conditions of varied secretory activity. *Endocrinology*, 55: 255, 1954.

11. Hökfelt, B. Selective depletion of the adrenaline content of the suprarenal gland by the injection of insulin in the cat. *Endocrinology*, 53: 536, 1953.
12. Gershberg, H. and Long, C. N. H. The activation of the adrenal cortex by insulin hypoglycemia. *J. Clin. Endocrinol.*, 8: 587, 1948.
13. Perlmutter, M. and Mufson, M. The hypoglycemic and eosinopenic response to insulin. *J. Clin. Endocrinol.*, 11: 277, 1951.
14. Gershberg, H., Fry, E. G., Brobeck, J. R. and Long, C. N. H. The role of epinephrine in the secretion of the adrenal cortex. *Yale J. Biol. Med.*, 23: 32, 1950.
15. Madison, L. L. Comparison of anterior pituitary-adrenal cortical stimulating effect of U. S. P. epinephrine, synthetic l-epinephrine and norepinephrine. *J. Clin. Investigation*, 29: 789, 1950.
16. Harrison, T. R. and Finks, R. M. Glucose deficiency as a factor in the production of symptoms referable to the cardiovascular system. *Am. Heart J.*, 26: 147, 1943.
17. Lukens, F. D. W., Spoont, S. and Barol, S. R. Hypoglycemia. *J. Clin. Endocrinol.*, 14: 248, 1954.

Pericardial Effusion in Generalized Scleroderma*

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SCLERODERMA had been long known to involve several systems[1] when Weiss et al.[2] presented convincing evidence that the same pathologic process involved the heart muscle as well. Previous studies had pointed out the occurrence of right ventricular enlargement in patients with advanced pulmonary disease.[3,4] Weiss et al. were the first to call attention to the clinical picture in scleroderma of intractable right- and left-sided congestive failure, enlarged triangular heart shadow with weak pulsations, and abnormalities of conduction in the electrocardiogram. These investigators considered the roentgen findings as suggestive of pericardial effusion. However, in the two postmortem examinations reported the striking finding was a flabby dilated heart with extensive replacement of muscle by fibrosis, and a relatively small amount of fluid, 120 and 340 ml., in the pericardial sac. Since then several other reports have been made of patients with sclerodermatous heart disease with similar findings. Of those reported by Mathiesen and Palmer,[5] Hurley, Case and Weber[6] and Biegelman, Goldner and Bayles[7] each series included one patient with hydropericardium at postmortem, associated with intractable congestive failure and extensive myocardial involvement. The quantity of fluid was 300, 100 and 800 ml., respectively, and was said to be clear and straw-colored but was not otherwise described. East and Oran,[8] and Goetz[9] found no significant fluid in their patients. Barritt and O'Brien[10] reported an attempt at paracentesis. Their patient had a large weakly pulsating heart shadow and was twice tapped unsuccessfully. In a recent review Leinwand, Duryea and Richter[11] stated that pericardial effusion rarely if ever occurs in scleroderma.

The following two cases of generalized scleroderma are presented to illustrate extensive pericardial effusion diagnosed and tapped antemortem. The first patient died within four months of diagnosis, three years after the onset of Raynaud's phenomena. The second patient is still alive ten months after the diagnosis was established and six years after onset of Raynaud's phenomena.

CASE REPORTS

CASE 1. F. P., a fifty-one year old Italian born housewife, entered the Presbyterian Hospital in August, 1947, because of swollen, painful finger joints and twenty-pound weight loss of one year's duration. Except for mild chronic cholecystitis, she was in good health.

Three years prior to admission, coincident with menopause, mild Raynaud's phenomena were noted. These continued without progression for two years, at which time swelling, pain and stiffness of the finger joints developed. In the year prior to admission the skin on the hands and feet became increasingly thick, taut, glistening and pigmented. For eight months the patient had difficulty swallowing and often vomited any sizable meal. She lost more than twenty pounds, was extremely weak and bedridden most of the time. No respiratory symptoms, chest pain, obvious fever or sweats were present and, with her very limited activity, no dyspnea or orthopnea. There was no cold intolerance, hoarseness or mental retardation.

Physical examination revealed a chronically ill woman weighing only ninety-four pounds, with vitiligo and pigmentation in the thickened, shiny, atrophic skin over the hands, arms, face, neck and thorax. The blood pressure was 110/60, pulse 80 and regular, respirations 18 per minute and temperature 100°F. There was no paradoxic pulse. The lungs were clear and the heart markedly enlarged to the right and left, with a faintly palpable impulse. The heart sounds were fair and a soft, apical systolic murmur was present. A_2 was greater than P_2. There was no rub. The liver was enlarged to three fingerbreadths below the costal margin and tender. The neck veins were flat but there was a two plus pitting ankle edema. The

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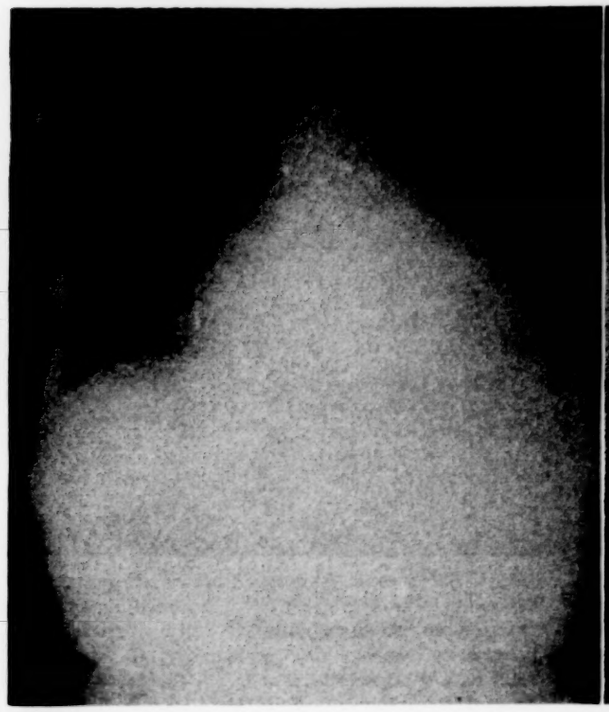

FIG. 1. Case 1. Film taken on admission shows markedly enlarged globular heart shadow with no pulmonary congestion.

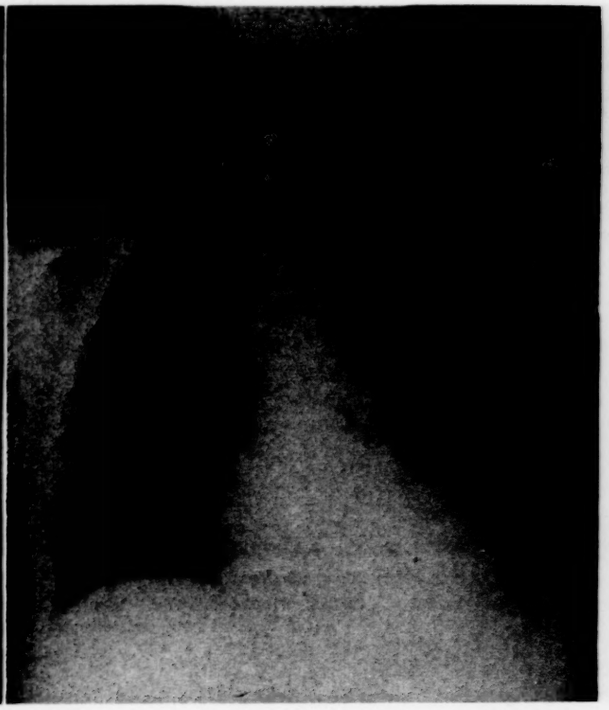

FIG. 2. Same case. Post-tap film showing pneumopericardium, a thin pericardial sac and a heart shadow of normal size.

remainder of the physical examination was within normal limits.

The significant laboratory data included a sedimentation rate of 120, a hemoglobin of 11.5 gm./100 ml. and a white cell count of 11,650 with normal differential. The urine was within normal limits and the blood urea nitrogen 8 mg./100 ml. A Kline test gave negative results. The stools were brown but consistently guaiac-positive. Electrocardiogram showed only low voltage. X-ray study of the gastrointestinal tract disclosed atony and dilatation of the esophagus. The stomach, duodenum, small intestine and colon were normal. The lung fields were clear and the vascular markings were not increased. The globular heart shadow was markedly enlarged (Fig. 1) and was noted to pulsate weakly.

Biopsy of the skin showed atrophy of the dermis and greatly thickened collagen bundles which gave the typical "fibrinoid" staining reaction.

The patient was thought to have generalized scleroderma involving the fingers, skin, heart and esophagus. Mild right- but no left-sided failure was present, although the neck veins were not full. Several venous pressures were technically unsatisfactory. The etiology of the pericardial effusion was not clear but the patient was not thought to have an infarction. There was no evidence of rheumatic fever and no pain or rub to suggest the "non-specific" type of pericarditis. No clinical findings of myxedema were evident but no laboratory study of thyroid function was made. She had low grade fever of 99.4° to an occasional maximum of 101°F. There was no evidence of tuberculosis in the lungs or kidneys. Tuberculin test was not done.

A diagnostic pericardial tap was performed, removing 550 ml. of cloudy yellow non-clotting fluid without any significant change in vital signs. The characteristics of the fluid are charted in Table 1. The cardiac silhouette decreased after tap but remained enlarged. A pneumopericardium was induced and subsequent x-ray of the chest showed that the pericardial sac was not thickened, and that the heart chambers were within normal limits. (Fig. 2.)

TABLE 1
CHARACTERISTICS OF THE PERICARDIAL FLUID

	Case F. P.	Case C. T.
Color	Straw	Straw
Clot	None	Slight clot
Amount removed	550 cc.	175 cc.
Specific gravity	1.022	1.020
Protein	5.9 gm. %	6.8 gm. %
White cells	37 per cu. mm.	4 per cu. mm.
Red cells	22 per cu. mm.	0
Bacterial culture	No growth	No growth
Culture for tuberculosis	No growth	No growth
Guinea pig inoculation	Not done	No evidence for tuberculosis

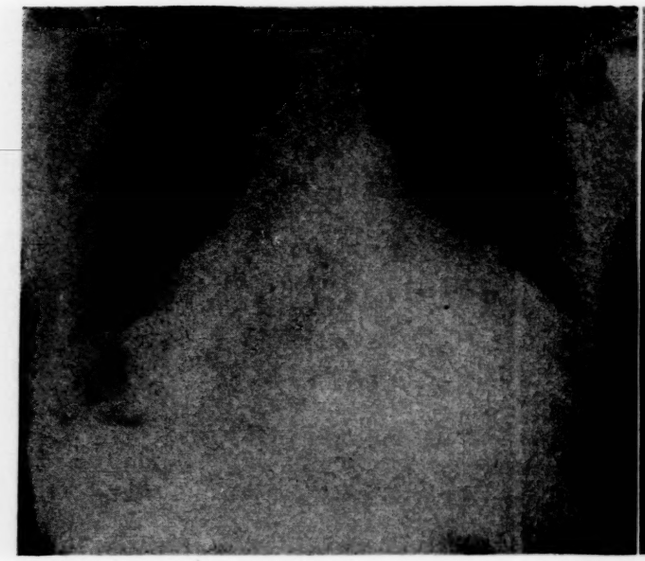

FIG. 3. Case II. Film taken at the time of second admission showing very large globular heart shadow and clear lung fields.

FIG. 4. Same case. Angiocardiogram. Film taken 2 seconds after dye injection showing filling of the superior vena cava, right auricle, right ventricle and pulmonary vessels. The pericardial effusion appears as a shadow which is much too large to be accounted for by myocardium.

The patient's subsequent course was downhill. She was given testosterone without any effect. Two months after discharge she returned with severe congestive failure, gallop rhythm, hepatomegaly, marked edema of the leg, hydrothorax and pulmonary congestion. She died suddenly during her admission workup. No postmortem examination was obtained.

CASE II. C. T., a thirty-six year old Negro housewife, was first seen at the Presbyterian Hospital in September, 1951, because of weakness and excessive menstrual flow. At that time she had had Raynaud's phenomena for three years and depigmentation, tightening and cracking of the skin of the hands for one year. In the six months prior to admission she had noted finger joint arthralgias, weakening of her hand grip and dyspnea after climbing one flight of stairs.

The temperature was 98.6°F., pulse 96 and regular, respirations 16 per minute. Blood pressure was 120/75. The skin showed areas of depigmentation on the finger joints and was thin, taut and shiny. Her hand grip was weak and the small muscles of both hands were moderately atrophied. Examination of eyes, ears, nose, throat, thyroid and lymph glands was normal. The lungs expanded well and were clear to percussion and auscultation. The heart was not enlarged and the sounds were of good quality with a soft apical systolic murmer. A_2 and P_2 were equal. On examination abdomen was found to be normal. The peripheral pulses were full and equal and there were no abnormal reflexes. Several uterine fibroids were felt on pelvic examination.

The significant laboratory data included a sedimentation rate of twenty-seven, white blood count of 10,500 with normal differential, hematocrit of 29 per cent, hemoglobin of 7.0 gm. per 100 ml. and red cell count of 3.07 million per cu. mm. Reticulocytes were 0.7 per cent and a bone marrow aspiration showed normal marrow. The Mazzini test gave negative results and urinalysis was within normal limits. A gastrointestinal series showed equivocal dilatation of the esophagus. Electrocardiogram and two meter heart film were within normal limits.

The patient was thought to have scleroderma involving skin and possibly the esophagus as well. Anemia was explained on the basis of blood loss from uterine bleeding. Hysterectomy was advised and performed. The patient did well postoperatively and was discharged on oral iron therapy in December, 1951. Two months later hemoglobin was 12.4 gm. per 100 cc. There was mild general remission. She was able to crochet and return to work.

The clinical picture did not change until March, 1954, when the patient returned to the clinic because of progressive dyspnea of four months' duration. She had had no gastrointestinal symptoms.

Physical examination revealed a temperature of 99.8°F. pulse of 96 with occasional premature contractions, respirations of 16 per minute and blood pressure of 115/75. She was comfortable lying flat and had no edema or dilated neck veins. The skin showed progression of sclerodermatous changes over the hands, shoulders, face and neck. The lungs were clear. The heart was markedly enlarged with a poorly transmitted impulse. The sounds were not distant but there was a systolic gallop and murmer. P_2 was greater than A_2 but was not pronounced. The

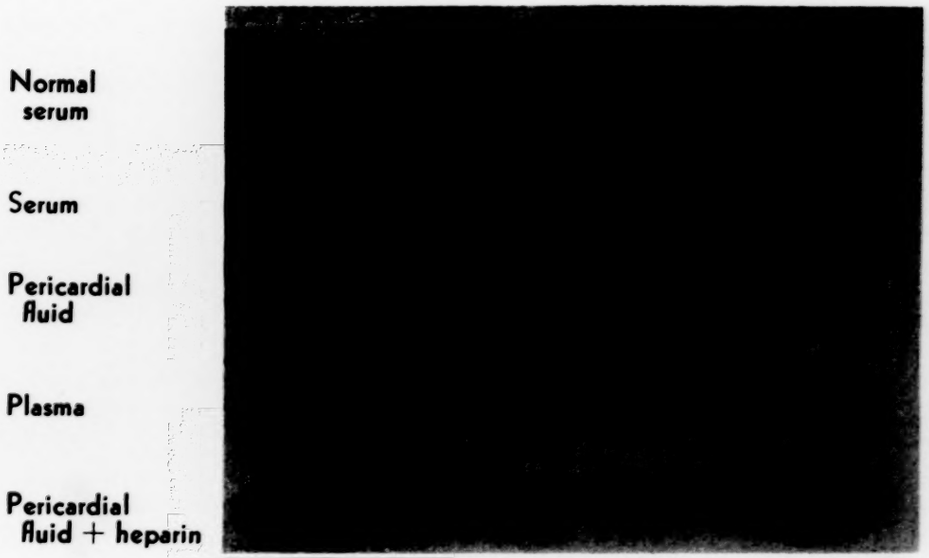

FIG. 5. Case II. Filter paper electrophoresis of patient's serum, plasma and pericardial fluid. The top pattern is a normal control. The patient has an elevated globulin in both blood plasma and pericardial fluid.

rest of the physical examination revealed nothing pertinent.

Laboratory study showed a sedimentation rate of thirty-seven, hematocrit of 36 per cent, white cell count of 8,000 with 61 per cent lymphocytes. The urine had 2+ protein. The blood urea nitrogen was 18 mg./100 ml., the serum albumin 4.0 and globulin 3.4 gm./100 ml., respectively, cholesterol 127 mg./100 ml. and radioactive iodine uptake 28 per cent at twenty-four hours. The serum protein bound iodine was 4.3 gamma/100 ml.

The major symptoms were marked weakness and angina necessitating bed-chair rest. Roentgen study revealed a large globular heart which pulsated poorly (Fig. 3) and a dilated atonic esophagus. The lung fields were clear and the venous pressure was 70 mm. of water. Electrocardiogram showed a normal P-R interval, prolongation of the intraventricular conduction time to 0.10 seconds and T wave inversions consistent with the pericardial effusion.

The presence of a huge, weakly pulsating heart shadow in the absence of any objective evidence of congestive failure suggested pericardial effusion rather than cardiac dilatation. An angiocardiogram was obtained prior to attempting a pericardial paracentesis. The study clearly demonstrated a 4 cm. border of cardiac shadow all the way around normal heart chambers. (Fig. 4.) A pericardial tap was done, and 175 ml. of clear, straw-colored fluid were removed for diagnostic purposes. The character of the fluid is described in Table I.

In her four weeks of hospitalization she showed no signs of tamponade. However, she had frequent angina and was too weak to walk. She was then transferred to another hospital for further care, without any drug therapy.

When seen in the clinic eight months later, she was somewhat improved symptomatically and was able to get about the house. She rarely ventured out, however, because of weakness and attacks of anginal pain only partially relieved by nitroglycerine. Laboratory study showed no important changes. Fluoroscopy revealed persistence of massive pericardial effusion. The patient was still without objective signs of congestive failure. However, there was evidence of further skin involvement and progression of gastrointestinal tract lesions to include the entire esophagus, stomach and small intestine.

COMMENTS

The first patient was not completely studied in that myxedema was not excluded by laboratory data, and the fluid was not inoculated into guinea pigs for evidence of tuberculosis. Unfortunately a postmortem examination was not obtained. However, there were no clinical signs or symptoms of myxedema. The thin pericardium and the finding of so few cells in a pericardial fluid containing a high concentration of protein suggests a non-infectious process. In the presence of extensive scleroderma elsewhere, the sclerodermatous etiology of the pericardial effusion is most likely. In the second case, the other known causes of effusion appear to have been excluded on both clinical and laboratory grounds.

It is of interest that patient C. T. had no evidence of congestive failure at the time of diagnosis and in an eight-month follow-up

period. Thus at physical examination the presence of a marked increase in cardiac dullness with a weakly palpable cardiac impulse first suggested the diagnosis of an effusion rather than dilatation.

The pericardial fluid obtained in the two patients was similar, each containing a high concentration of protein with few cells and small amounts of fibrinogen. (Table 1.) It is not possible to conclude anything about the mechanism involved in the formation of the fluid but several observations are important in this regard. The pericardium at postmortem in cases of scleroderma involving the heart has been described as fibrous.[12] In patient C. T. we were able to obtain paper electrophoresis patterns of plasma and pericardial fluid. These were virtually identical in pattern and concentration except for fibrinogen. (Fig. 5.) Perhaps the fibrotic change that occurs in the pericardium is the basis for this similarity.

The prognosis as to disability seems to depend primarily on the severity of the cardiac rather than the pericardial involvement. In patient C. T. the effusion itself does not appear to be of particular clinical significance other than the fact that pericardial involvement appears to occur only in the severe, generalized disease. The fact that patient C. T. did not develop tamponade despite extensive effusion may be attributed to the thin, fibrotic, distensible pericardial sac, and the relatively few cells in the exudate.

SUMMARY

1. Two patients with pericardial effusion apparently due to involvement of the pericardium in scleroderma are described.

2. The pericarditis associated with scleroderma may have a protracted course and may not significantly contribute to disability over long periods of time.

3. The fluid in both cases was similar, characterized by high protein, low cell count, absence of blood and negative culture.

4. In one case it was shown that the electrophoretic pattern of the heparinized pericardial fluid and the plasma was virtually identical.

REFERENCES

1. Lewin, G. and Heller, J. Die Sclerodermie. Berlin, 1895. A. Hirschwald.
2. Weiss, S., Stead, E. A., Warren, J. V. and Baily, O. T. Scleroderma heart disease. *Arch. Int. Med.*, 71: 749, 1943.
3. Matusi, S. Über die Pathologie und Pathogenese von Sclerodermia universalis. *Mitt. a. d. med. Fakult. d. k. Univ. zu Tokyo*, 31: 55, 1924.
4. Kraus, E. J. Zur Pathogenese der diffusen Sclerodermie. *Virchows Arch. f. path. Anat.*, 253: 710, 1924.
5. Mathisen, A. K. and Palmer, J. D. Diffuse scleroderma with involvement of the heart. *Am. Heart J.*, 33: 366, 1947.
6. Hurly, J., Coe, J. and Weber, L. Scleroderma heart disease. *Am. Heart J.*, 42: 758, 1951.
7. Beigelman, P. M., Goldner, F. and Bayles, T. B. Progressive systemic sclerosis (scleroderma). *New England J. Med.*, 249: 45, 1953.
8. East, T. and Oram, S. The heart in scleroderma. *Brit. Heart J.*, 9: 167, 1947.
9. Goetz, R. H. The heart in generalized scleroderma. *Angiology*, 2: 555, 1951.
10. Barritt, D. W. and O'Brien, W. Heart disease in scleroderma. *Brit. Heart J.*, 14: 421, 1952.
11. Leinwand, I., Duryee, A. W. and Richter, M. N. Scleroderma. *Ann. Int. Med.*, 41: 1003, 1954.
12. Spain, D. M. and Thomas, A. C. The pulmonary manifestations of scleroderma: an anatomic-physiological correlation. *Ann. Int. Med.*, 32: 152, 1950.

Infectious Mononucleosis of the Central Nervous System*

Demonstration of Atypical Lymphocytes in the Cerebrospinal Fluid

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INVOLVEMENT of the central nervous system in the course of infectious mononucleosis is being recognized more frequently. In 1948 Bernstein and Wolff[1] summarized reports of thirty-four patients with nervous system disease due to infectious mononucleosis and added an additional case report. Subsequently, a number of such cases have been reported.

All valid cases reported to date have been characterized by evidence of systemic involvement with accompanying hematologic and serologic changes. Although heterophil antibody has been present in the blood of all patients tested, it has rarely been demonstrated in the spinal fluid. This antibody was sought in the spinal fluid of six of thirty-five patients reviewed in 1948, but it was found in none. In subsequent case reports heterophil antibody has been found in the spinal fluid of several.[2–4] Silberstein et al. found six patients with infectious mononucleosis who had antibody in the spinal fluid, only one of whom had signs of central nervous system involvement.[5] They found three positive tests among 688 examinations of spinal fluid from patients not known to be suffering from the disease.

The cytology of the spinal fluid in infectious mononucleosis involving the nervous system has not been described. There have been no reports of atypical lymphocytes in the spinal fluid. In the case to be reported the diagnosis was obscure until the cytology of the spinal fluid was studied. The appearance in the spinal fluid of atypical lymphocytes led to the subsequent serologic tests and their provocative results.

CASE REPORT

Patient J. T., a forty year old man, had been continuously hospitalized for six years because of a paranoid schizophrenic reaction. His psychiatric condition had proceeded to a point where a bilateral prefrontal leukotomy was considered to be indicated; this was performed on January 20, 1954. Prior to operation the patient had had an extensive medical examination and laboratory work-up which revealed no evidence of systemic disease. However, on January 6th he had been examined because of pain in the right side of the neck, right shoulder and right upper extremity. On January 14th the right radial reflex was found to be absent and a diagnosis of extruded nucleus pulposus of a cervical intervertebral disc was considered briefly.

Shortly after surgery a fever of 101°F. was noted, ascribed at the time to some operative bleeding. On January 22nd it was noted that the patient's neck was stiff and that a positive Brudzinski sign was present. Except for the previously described absent right radial reflex, no other neurologic abnormalities were found. The patient appeared to be mentally dull when compared to his preoperative and immediate postoperative state. By January 24th the fever had increased to 103°F. and the neck rigidity was marked. A white blood count at this time showed 12,000 cells per cu. mm., with 82 per cent polymorphonuclear leukocytes. Fever in the range of 103°F. and increasing meningeal signs continued for the next week. Examination of the spinal fluid on January 26th showed initial pressure of 290 mm. of water, xanthochromia, cell count of six red blood cells and 551 white blood cells, and 59 mg. per cent of protein. By February 3rd his fever had fallen to 101–102°F., the neck signs were still positive, and the deep tendon reflexes were sluggish. The patient was disoriented, confabulated and still appeared lethargic. By the following day neck rigidity was diminishing and the deep reflexes were very easily obtained. Fever had decreased to 100–101°F. Although depressed, the patient was considerably brighter then he had been earlier in the postoperative course. By February 6th only slight neck rigidity remained and temperature had become normal.

Cerebrospinal fluid taken on February 8th revealed 387 white blood cells, 78 per cent of which were

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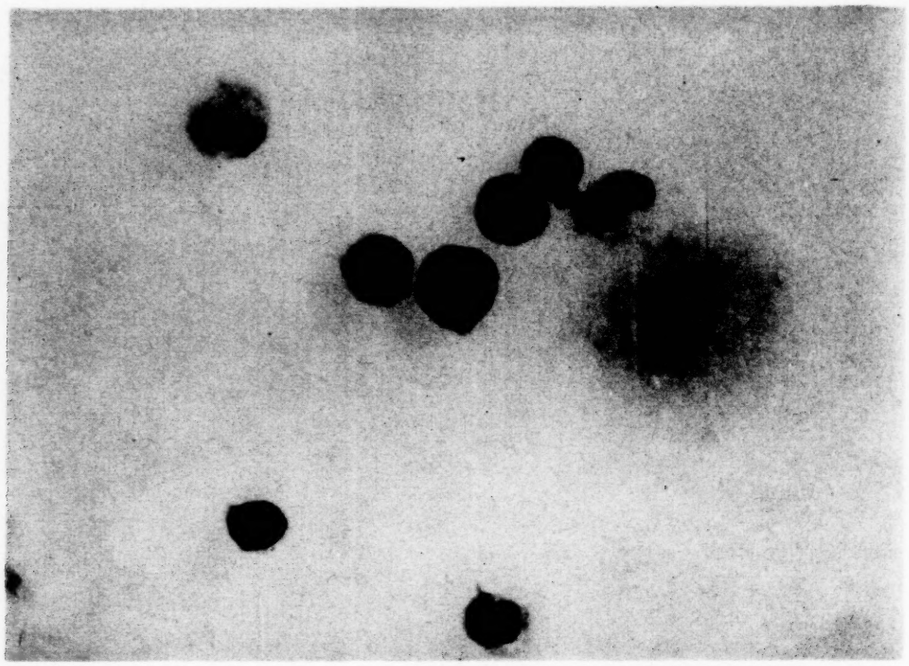

FIG. 1. Atypical lymphocytes in cerebrospinal fluid (smear of sediment, Wright stain).

lymphocytes. Microscopic examination of these cells showed that 40 per cent were atypical lymphocytes. (Fig. 1.) On February 9th the first of the determinations of heterophil antibody in the blood was done. An antibody strongly resembling that of infectious mononucleosis was found in significant titer. (Table I.) On February 10th another examination of cerebrospinal fluid was made. This fluid was clear, under normal pressure, and had a protein content of 114 mg. per cent; it also contained atypical lymphocytes. Heterophil antibody was found in spinal fluid obtained on this date and the differential absorption tests indicated that it was the antibody of infectious mononucleosis. Subsequent tests of heterophil antibody in the spinal fluid and blood are described in Table I. By this time the patient appeared to be improving rapidly. He was mentally brighter and there were no abnormal neurologic signs or fever. By February 12th the patient was well oriented, cheerful and appeared to have recovered from the meningitis.

TABLE I

HETEROPHIL ANTIBODY IN BLOOD AND SPINAL FLUID OF CASE REPORTED

1954	Blood					Cerebral Spinal Fluid		
	Not Absorbed	Guinea Pig Kidney	Ox RBC	Rabbit RBC	Human RBC	Not Absorbed	Guinea Pig Kidney	Ox RBC
2/9	1:448	1:224	1:28	....	.....	.....	....	....
2/10	.....	.....	....	....	.....	1:112	1:56	1:14
2/16	1:896	1:112	1:56	....	.....	1:56	1:28	1:14
2/23	1:224	1:56	1:56	....	.....	1:14	1:7	1:7
3/4	1:112	1:28	1:7	1:14	.....	1:7	1:7	1:7
3/11	1:112	1:14	1:7	1:7	.....	.....	....	....
3/15	1:112	1:28	1:7	1:7	.....	.....	....	....
3/24	1:112	1:28	1:28	1:28	.....	.....	....	....
3/31	1:112	1:28	1:14	1:14	1:112	.....	....	....
4/13	1:112	1:14	1:14	1:28	1:112	.....	....	....
6/10	1:112	1:7	1:14	1:56	1:112	.....	....	....

However, abnormalities in the spinal fluid persisted until March 4th. The pain in the right shoulder and upper extremity persisted for the same length of time. At this time the right radial reflex was found to have returned with normal activity.

Following the observation of the atypical lymphocytes in the spinal fluid and the subsequent finding in the blood and spinal fluid of heterophil antibody resembling the antibody of infectious mononucleosis, numerous attempts were made to detect other evidence of infectious mononucleosis. Repeated examinations of the peripheral blood were made during the preoperative and postoperative periods, none of which showed hematologic evidence of infectious mononucleosis. A series of liver function tests done on March 15th showed no abnormal results. A week prior to operation the same group of tests had been done. The cephalin flocculation test was 3 plus, which was the only questionable abnormality. Repeated and careful examinations for physical signs of infectious mononucleosis were made but none were found. The patient recovered completely from his complicated postoperative course and two and a half months after operation he was released from the hospital on trial visit.

FURTHER STUDIES

Fever and meningeal signs of short duration are occasionally seen following bilateral prefrontal leukotomy, especially in those cases complicated by bleeding. The duration of these signs was unusually long in this patient. To determine if leukotomy alone could account for the antibody changes observed, fourteen additional patients subjected to leukotomy were also studied. Ten of these fourteen patients were tested prior to operation and antibody was not then noted in the blood. Following operation, heterophil antibody titers of 1:14 or more were noted in blood specimens of eleven patients. Most of the titer levels were 1:56, but in two patients a level of 1:224 was obtained. The differential absorption tests were similar in these patients. After absorption on guinea pig kidney, titers were reduced to 1:7 or less in all patients but one. In this patient four-tube absorption occurred to a titer of 1:14. Absorption on ox erythrocytes caused a lesser degree of absorption in eight patients than occurred from absorption with the guinea pig kidney. Exposure to rabbit erythrocytes caused significant absorption of antibody in two patients. The cerebrospinal fluid was tested in eight patients following surgery. One patient who had a complicating brain abscess showed a spinal fluid titer of 1:7. Another patient, whose spinal fluid was contaminated by blood from a traumatic tap, had antibody present in the undiluted specimen.

The antibody found in these eleven patients appeared one to three weeks following surgery and remained for as long as three months. We have not been able to discover this antibody in mixtures of normal blood and spinal fluid, nor in mixtures of blood and brain tissue obtained by suction at operation. This antigen does not appear to be present in normal human brain tissue; it is not absorbed by exposure to such tissues.

COMMENT

The case reported is presented as involvement of the central nervous system by infectious mononucleosis. The discovery of a lymphocytic meningitis characterized by abnormal lymphocytes in the spinal fluid, and the presence of an antibody in the blood and spinal fluid which appeared to be that of infectious mononucleosis, is difficult to explain on any other basis. The transitory radiculitis noted before operation may suggest that the disease was present in mild form prior to surgery. In such a case, surgery might have produced an exacerbation of the disease, a suggestion others have also made.[6]

To ascribe the course of the case reported to the operation alone would be difficult. Study of fourteen additional patients subjected to leukotomy revealed significant differences from the patient reported. First, the clinical course was entirely different, except in the patient with a complicating staphylococcal brain abscess. Second, the heterophil antibody titers were lower in the blood of these patients. Third, heterophil antibody appeared in the spinal fluid of these patients only when the blood-brain barrier was affected or when the spinal fluid was contaminated grossly by blood containing the antibody. Fourth, the differential absorption patterns of the antibody found in these patients indicated that it was either Forssman or serum sickness type. All of the reactions noted in these patients have been described in normal persons with low initial antibody titers.[7] It may be that these antibodies arose anamnestically following the operation.

Until recently, all reports of central nervous system involvement in infectious mononucleosis have been associated with systemic, serologic and hematologic evidence of the disease. A

recent report in which the presenting and clinically important findings were in the nervous system raises the possibility that the etiology of some cases of benign lymphocytic meningitis might be explained if heterophil antibody tests were performed routinely in these patients.[4] This possibility is given additional support by the report of a blood picture indistinguishable from that of infectious mononucleosis in two patients with benign lymphocytic meningitis.[8] Tidy has called attention to the fact that the mononucleosis in the blood and the formation of agglutinins are associated with development of the glandular enlargement and not with the neurologic features of infectious mononucleosis.[9] If these two clinical manifestations do not appear simultaneously, the hematologic and serologic changes might not be present when the neurologic manifestations are evident.

It seems probable that involvement of the central nervous system by infectious mononucleosis can occur with little evidence of general systemic infection. The failure to detect such instances may be due to these reasons: (1) infectious mononucleosis is not often considered in the differential diagnosis of cases of lymphocytic meningitis of obscure etiology, (2) heterophil antibody determinations are seldom made on the blood and spinal fluid of these patients and (3) the detailed cytology of the spinal fluid has not been studied.

SUMMARY

A case is reported which is thought to represent an instance of involvement of the central nervous system by infectious mononucleosis without other clinical signs of the disease. This patient had clinical features of a lymphocytic meningitis following prefrontal leukotomy. Detailed study of the cytology of the spinal fluid revealed a high proportion of the lymphocytes to be atypical and of the type seen in infectious mononucleosis. Heterophil antibody was subsequently found in significant titers in the blood and spinal fluid. When at highest concentration, this antibody had differential absorption tests indicating that it was the antibody of infectious mononucleosis. No atypical lymphocytes were found in the blood.

REFERENCES

1. Bernstein, T. C. and Wolff, H. G. Involvement of nervous system in infectious mononucleosis. *Ann. Int. Med.*, 33: 1120, 1950.
2. Freedman, M. J., Odland, L. T. and Cleve, E. A. Infectious mononucleosis with diffuse involvement of nervous system: report of a case. *Arch. Neurol. Psychiat.*, 69: 49, 1953.
3. Raftery, M., Schumacher, E. E., Jr., Grain, G. O. and Quinn, E. L. Infectious mononucleosis and Guillain-Barré syndrome; report of three cases. *Arch. Int. Med.*, 93: 246, 1954.
4. Natzke, R. H. and Whiting, E. G. Meningoencephalitis due to infectious mononucleosis. *California Med.*, 81: 343, 1954.
5. Silberstein, J. K., Bernstein, T. C. and Stern, T. Demonstration of heterophile antibodies in cerebrospinal fluid from patients with infectious mononucleosis. *J. Lab. & Clin. Med.*, 33: 1204, 1948.
6. Unger, H. E. and Lyons, A. S. Infectious mononucleosis and appendicitis. *New York State M. J.*, 54: 1942, 1954.
7. Leibowitz, S. Heterophile antibody in normal adults and in patients with virus hepatitis. *Am. J. Clin. Path.*, 21: 201, 1951.
8. Wolf, P. Significance of proplasmocytes and atypical plasma cells in the benign lymphocytic meningitis syndrome. *Blood*, 9: 971, 1954.
9. Tidy, H. Glandular fever. *Brit. M. J.*, 2: 436, 1952.

Dextroposition of the Heart Simulating Congenital Dextrocardia*

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DISPLACEMENT of the heart to the right may be produced by a number of congenital and acquired lesions. These include eventration of the diaphragm, hiatus hernia, congenital absence of the right lung, atelectasis, pleural effusion, pneumothorax and obstructive emphysema. If marked, the displacement may readily be mistaken for congenital dextrocardia.

We have recently observed a patient in whom eventration of the diaphragm displaced the heart so that the roentgen appearance was suggestive of dextrocardia.

CASE REPORT

J. C., a sixty-five year old man, was admitted to the Philadelphia Veterans Administration Hospital in an advanced state of cachexia and dehydration. In the initial examination of the heart the apex beat was found to be in the fifth interspace in the right midclavicular line. The cardiac rhythm was grossly irregular, with a rate of 120 per minute. The blood

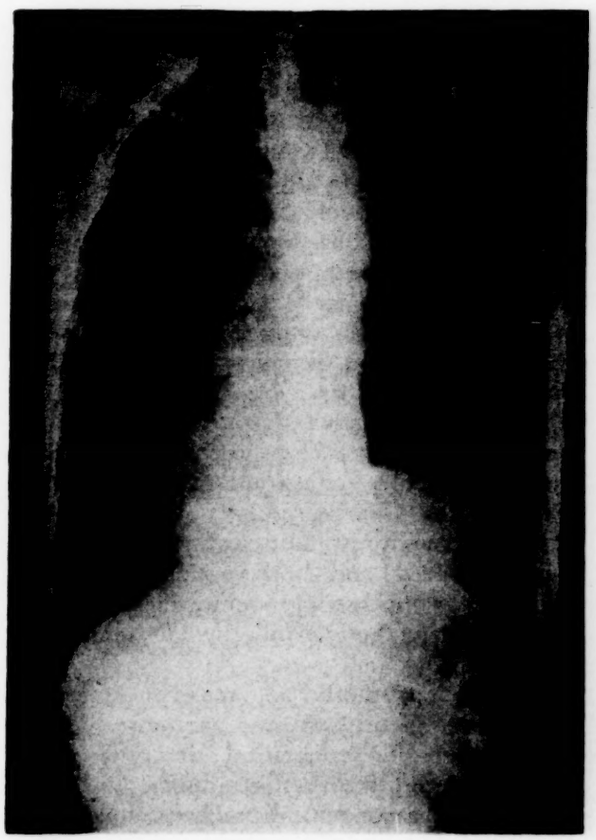

FIG. 1. Chest roentgenogram showing marked cardiac displacement to the right simulating dextrocardia.

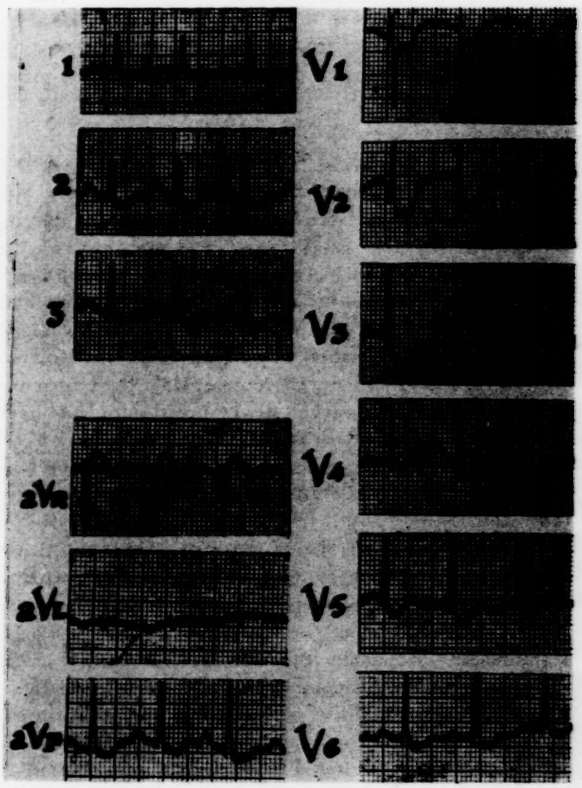

FIG. 2. Electrocardiogram of patient J. C. showing evidence of cardiac displacement in the precordial lead pattern but none of the features of congenital dextrocardia.

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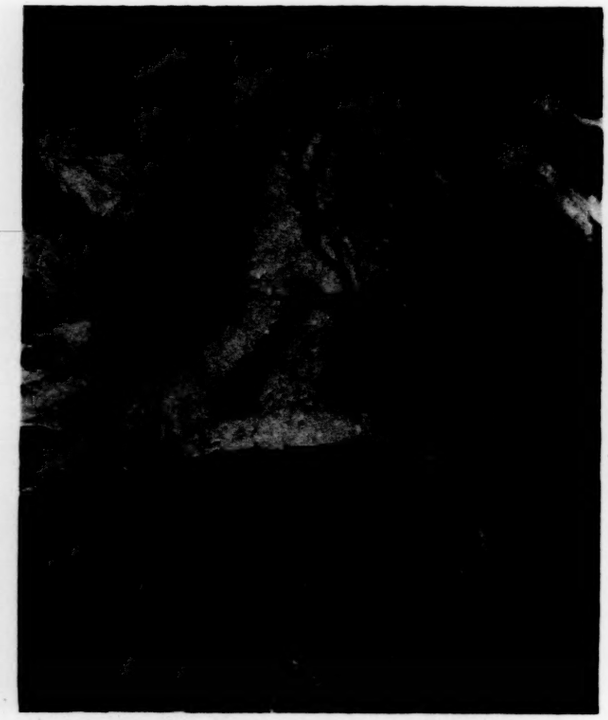

FIG. 3. Heart *in situ* showing its position in the right hemithorax. Note normal origin and course of the great vessels.

pressure was 130 systolic, 90 diastolic. An x-ray of the chest was reported to show dextrocardia with a left aortic arch. Either phrenic paralysis on the left or eventration of the diaphragm was thought to be present. (Fig. 1.) An electrocardiogram showed sinus tachycardia with many auricular premature beats. Two days later the rhythm was regular at a rate of 108 per minute. Neither tracing showed the electrocardiographic features of congenital dextrocardia (Fig. 2), suggesting marked dextroposition of the heart rather than true dextrocardia.

Despite supportive therapy the patient's course was progressively downhill and he died on the sixth hospital day.

Necropsy was performed and revealed anatomic distortion of the thoracic contents because of eventration of the left leaf of the diaphragm which had permitted the left lobe of the liver and a portion of the stomach to lie within the left pleural cavity. This resulted in upward displacement of the left lower lobe, and displacement of the pericardium and its contents to the right. The right border of the heart was approximately 2 cm. lateral to the right mid-clavicular line and the left border of the heart was in the midline. (Fig. 3.) The heart weighed 340 gm. There was some dilatation of the right chambers but all of the chambers were structurally normal and in normal relationship to each other. All of the septa were intact. The aorta had a normal origin, course and distribution.

FIG. 4. View showing eventration of the left diaphragm with displacement of the lung upward and the heart to the right.

Although the right leaf of the diaphragm was normal, the left leaf apparently lacked visible muscle fibers and consisted of a thin, translucent, glistening membrane. (Fig. 4.)

COMMENTS

The differentiation between dextroposition of the heart and dextrocardia can be of considerable importance. In some cases of cardiac displacement no treatment is necessary or feasible. When cardiac displacement is due to acquired acute lesions, such as pleural effusion, atelectasis, pneumothorax or obstructive emphysema, the need for treatment is self evident even if there are no symptoms present due to the mediastinal shift. Equen and his co-workers[1] reported two infants who were hospitalized because of progressive dyspnea, cyanosis and fever. In both cases chest roentgenograms revealed a picture consistent with congenital dextrocardia. The presence of obstructive emphysema with resultant cardiac displacement was recognized and both patients recovered following bronchoscopic removal of a non-opaque foreign body from the left main stem bronchus.

Tension pneumothorax, massive atelectasis and massive pleural effusion are more likely to be mistaken for congenital dextrocardia in infants and children than in adults.

Some of the congenital malformations which displace the heart are not amenable to therapy. Others, such as eventration of the diaphragm and hiatus hernia, can be corrected surgically if they produce symptoms.

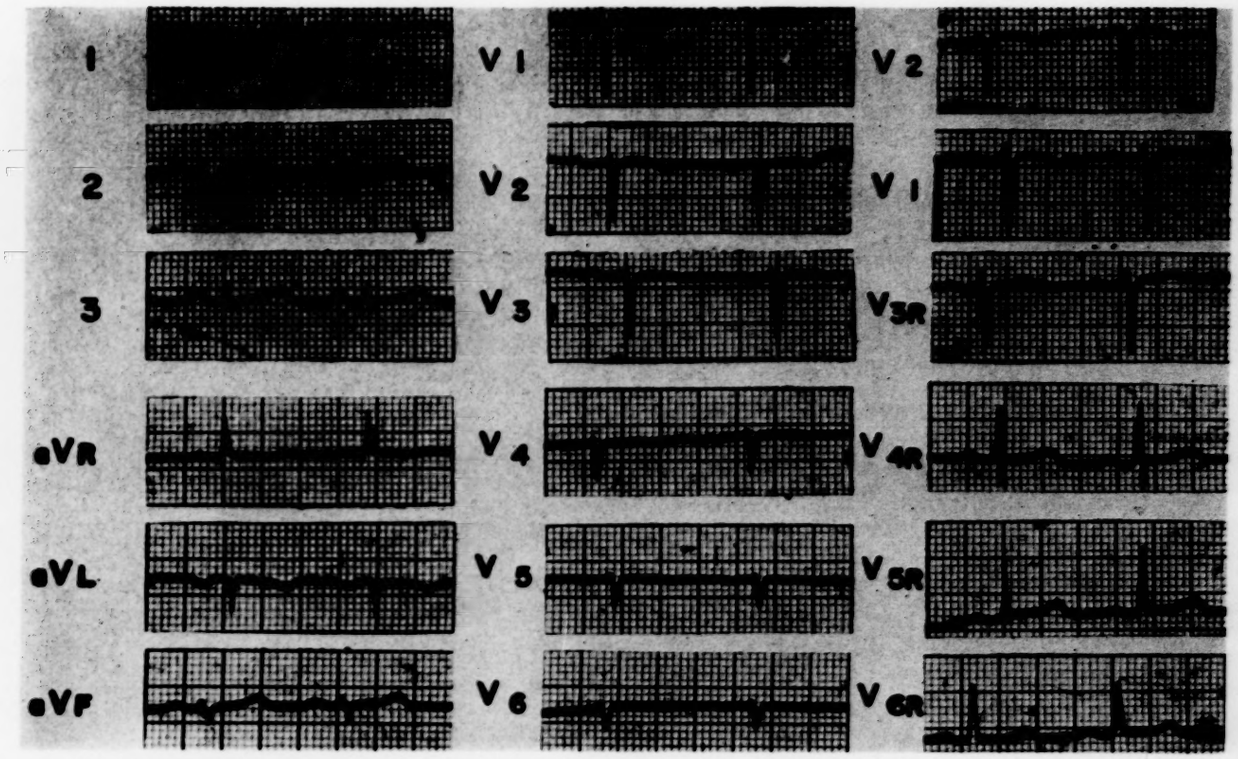

FIG. 5. The electrocardiogram in congenital dextrocardia. Note normal progression of chest leads to the right.

Eventration of the diaphragm may occur either as a solitary lesion or in association with absence of the right lung, and may produce marked cardiac displacement.[2] Sirry[3] found ten cases of eventration in 50,000 chest x-rays, an incidence of only 0.02 per cent. In nine of the ten cases the left leaf of the diaphragm was involved, with pronounced "dextrocardia" in four and some displacement of the cardiac silhouette in four others. Beck and Motsay[4] state that the symptoms of eventration of the diaphragm are due to pulmonary compression and mediastinal shift. Children or infants with this malformation may have dyspnea and cyanosis as presenting symptoms. Recognition and surgical correction of the lesion may be life-saving. Although differentiation of eventration of the diaphragm from massive hiatal hernia may be difficult, it is of more than academic interest since the surgical approach to the two lesions may be quite different.

Sirry[3] reported five patients in whom the left diaphragmatic leaf was elevated for reasons other than the presence of eventration of the diaphragm. These included two cases of phrenic nerve paralysis, one of diaphragmatic hernia, one of pneumoperitoneum and one of subphrenic abscess. Cardiac displacement was either slight or absent in these cases.

Other forms of "acquired dextrocardia" are described by Hedblom[5] who reported cardiac displacement in ten patients with tuberculosis, emphysema, pulmonary suppuration and pulmonary fibrosis.

Displacement of the heart must be differentiated from both true congenital dextrocardia and incomplete rotation of the heart (dextroversion).[2,6,7] In dextrocardia the primitive cardiac loop rotates in a direction opposite from the normal direction. This results in a heart that is a "mirror image" from left to right but is anatomically normal unless there are other associated malformations. It is believed that isolated dextrocardia is often associated with other congenital malformations of the heart while dextrocardia with complete situs inversus viscerum is usually structurally normal. The electrocardiogram in dextrocardia is pathognomonic.[6,8] Lead I is a mirror image of the normal, leads II and III are reversed, leads aVR and aVL are reversed, and the precordial leads show the normal progression to left ventricular potentials on the right side of the chest but not on the left. (Fig. 5.) This electrocardiographic

pattern is not seen in either incomplete rotation or cardiac displacement.

The differentiation of cardiac displacement from incomplete rotation of the heart may be more difficult. In incomplete rotation the cardiac chambers are in the normal relationship to each other but the heart is rotated counterclockwise so that the right atrium and right ventricle are to the right and posterior, while the left ventricle forms the major portion of the anterior cardiac wall. The apex of the heart is directed to the right. Since the electrocardiogram in this malformation does not differ from that of the displaced heart, differentiation must rest upon the identification of the factor or factors producing the displacement, and upon the absence of other evidence of congenital heart disease. Clinical differentiation may be impossible if incomplete rotation of the heart and conditions which might result in cardiac displacement coexist.

SUMMARY

1. A case of marked cardiac displacement due to eventration of the diaphragm, with the roentgen appearance of dextrocardia, is presented.

2. Displacement of the heart (dextroposition) may simulate congenital dextrocardia or incomplete rotation of the heart (dextroversion).

3. Differentiation of these conditions, although difficult, may be of considerable clinical importance.

4. True congenital dextrocardia can be identified by means of the pathognomonic electrocardiographic pattern. Congenital dextroversion may be difficult to differentiate from dextroposition except by identification of the factor or factors producing the displacement of the heart.

REFERENCES

1. Equen, M., Buckner, F., Roach, G. and Brown R. Dextrocardia secondary to foreign body in the left bronchus. *South. M. J.*, 45: 16, 1952.
2. Schwedel, J. B. Clinical Roentgenology of the Heart, pp. 345–347; 358–363. New York, 1946. Paul B. Hoeber.
3. Sirry, A. Radiological study of eventratio diaphragmatica. *J. Egyptian M. A.*, 37: 261–301, 1954.
4. Beck, W. C. and Motsay, D. S. Eventration of the diaphragm. *Arch. Surg.*, 65: 557–563, 1952.
5. Hedblom, G. A. Acquired dextrocardia. *Arch. Surg.*, 18: 349–364, 1929.
6. Taussig, N. B. Congenital Malformations of the Heart, pp. 499–513. New York, 1947. Commonwealth Fund.
7. Zdansky, E. Roentgen Diagnosis of the Heart and Great Vessels, pp. 309–320. New York, 1953. Grune & Stratton.
8. Burchell, H. B. The electrocardiogram in congenital heart disease. *M. Clin. North America*, 33: 1157–1175, 1949.

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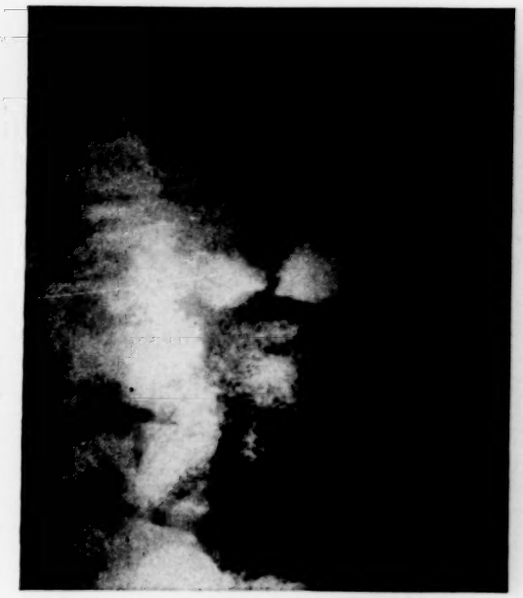
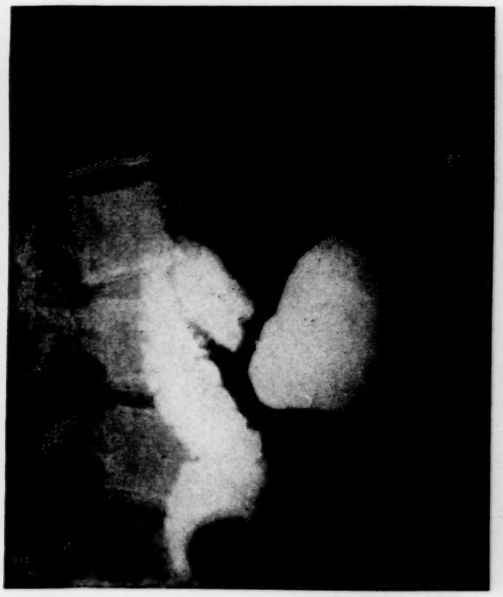


Basic film: pronounced hypermotility of stomach and bulb; diagnosis not possible.



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*Roentgenograms and case history courtesy of I. Richard Schwartz, M.D., Kings County Gastrointestinal Clinic, Brooklyn, N. Y.

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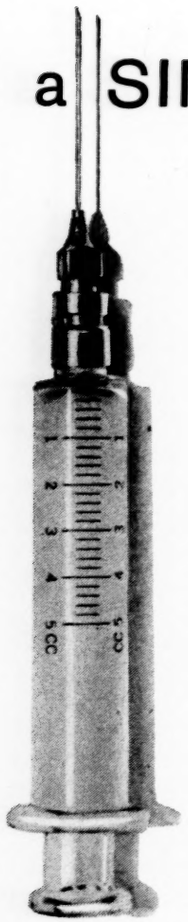

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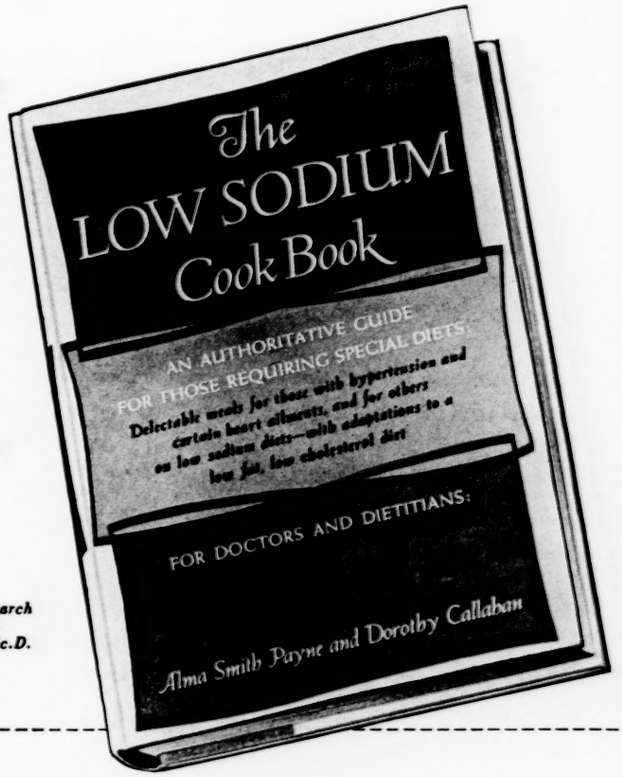

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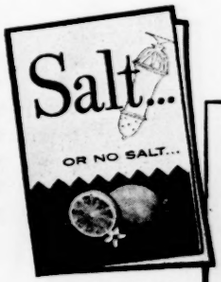

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Bibliography

1. Anonymous. 2. Rosenthal, P.: Geriatrics 10:382 (August) 1955. 3. Lansing, A. I.: Symposium on Problems of Gerontology, National Symposium Series No. 9 (August) 1954. 4. Mason-Hohl, E.: Quoted in W. Va. Med. J. 51:16 (January) 1955. 5. Rosenberg, H. R., et al.: Arch. Biochem. and Biophys. 49:263, 1954. 6. Bricker, M., Mitchell, H. H. and Kinsman, G. M.: J. Nutrition 30:269, 1945. 7. Masters, W. H. and Ballew, J. W.: Geriatrics 10:1, 1 (January) 1955.

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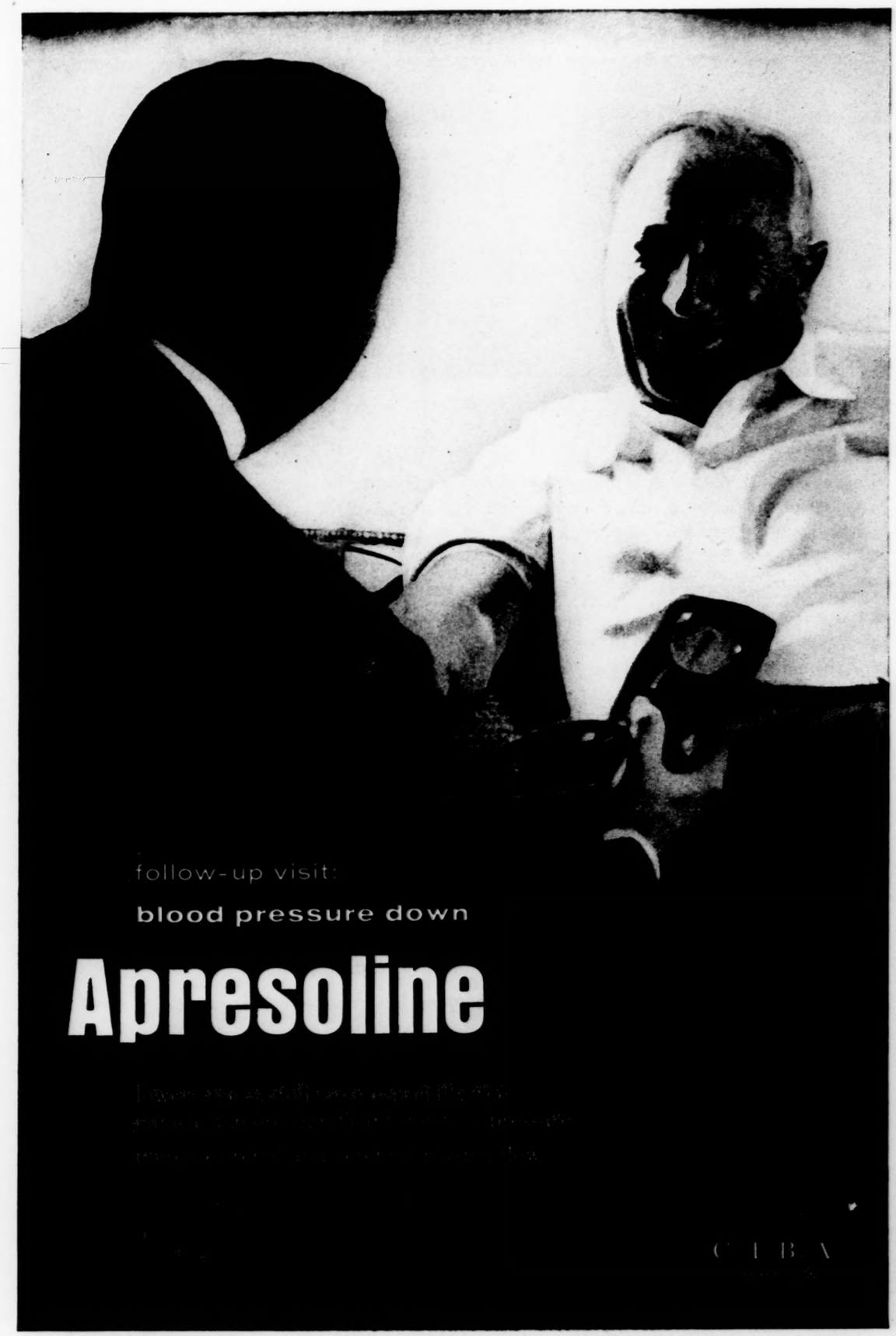
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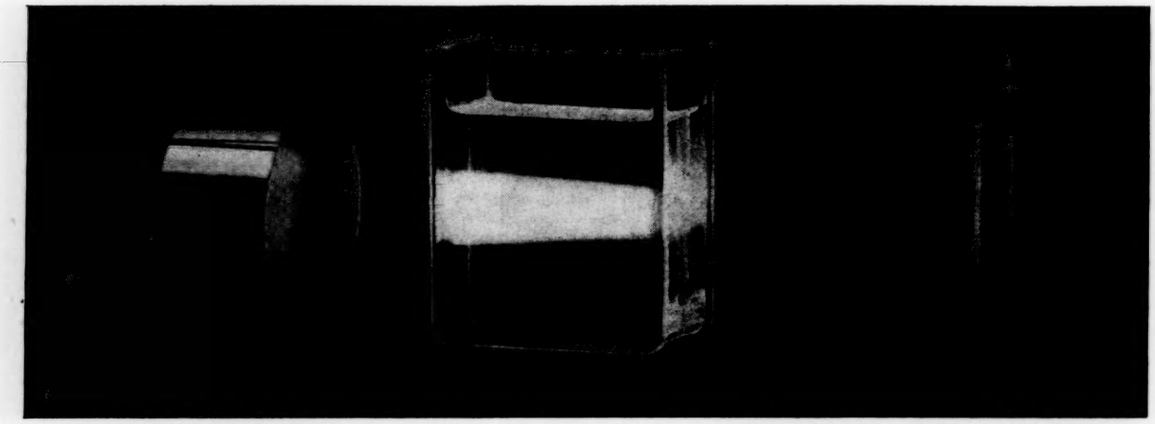

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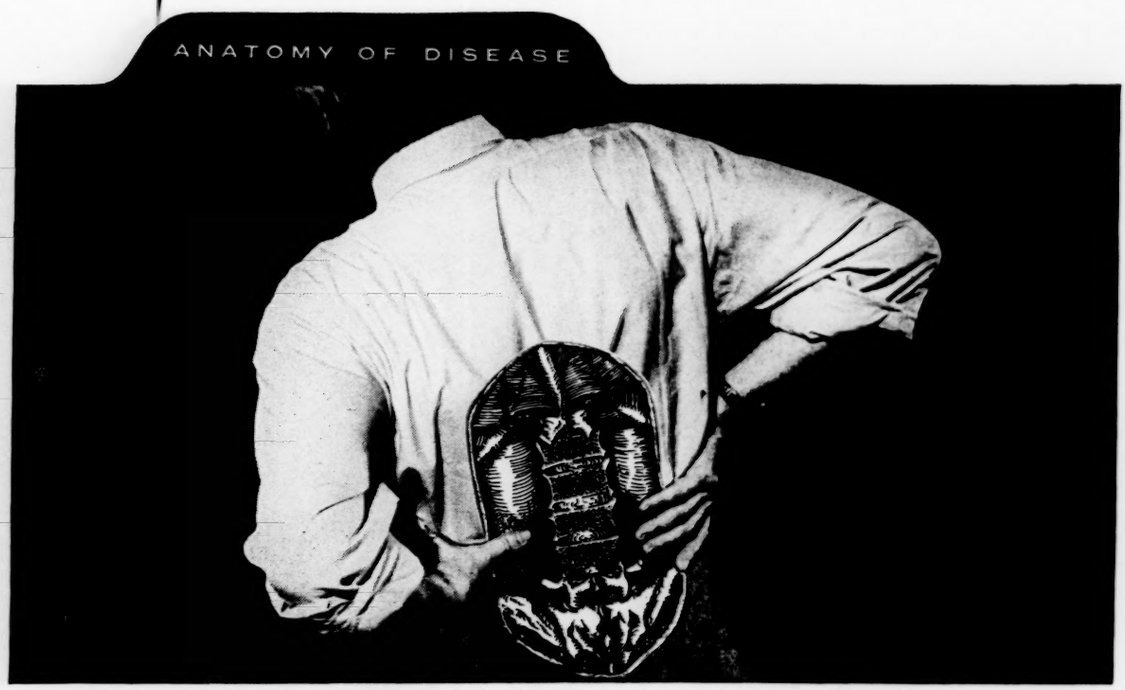

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Calories	379	13

*Berryman, G. H.: Obesity—A Brief Review of the Problem, Metabolism 3:544 (Nov.) 1954.

**Percentages of daily allowances for fairly active man 45 years of age, 67 inches in height, and weighing 143 pounds: Recommended Dietary Allowances, Washington, D.C., National Academy of Sciences—National Research Council Publication 302, 1953.

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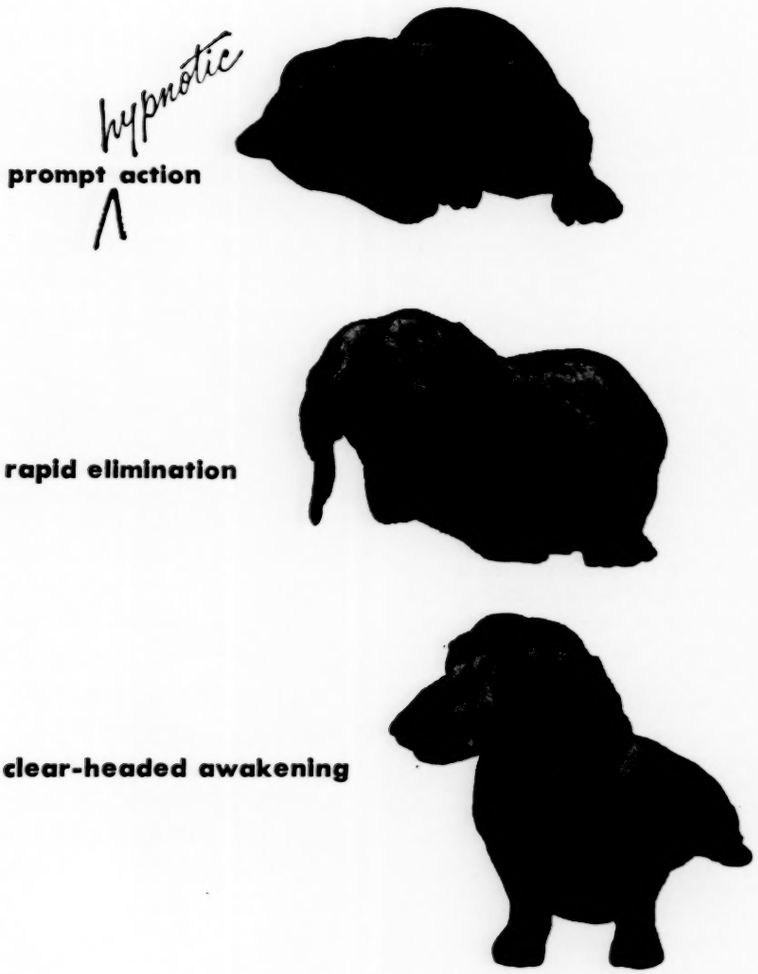

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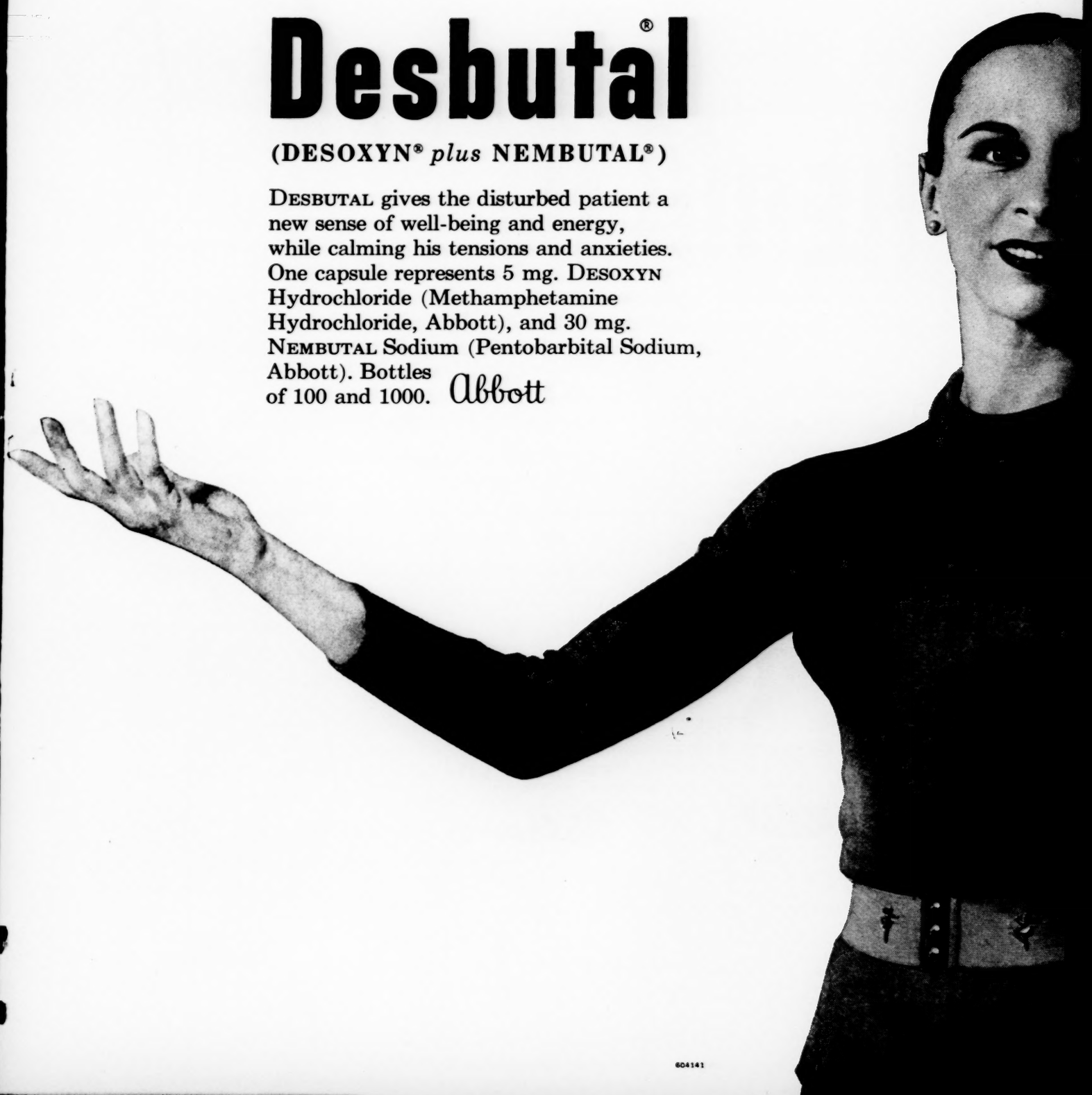

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Breneman, G. M., and Priest, E. McC.: Am. Heart J. 50:129-35, 1955.

". . . . . *phenindione seems to be a more satisfactory anticoagulant at this time.*"

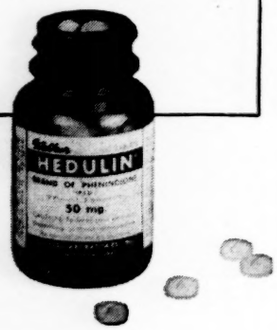
Wood, J. E., Jr.; Beckwith, J. R., and Camp, J. L.: J.A.M.A. 159:635, 1955.

HEDULIN permits dependable prothrombin control with little risk of dangerous fluctuation

(Not a Coumarin Drug)

DOSAGE 4 to 10 tablets (200 to 500 mg.) initially, half in the morning, and half at night; maintenance dosage (on basis of prothrombin determination daily for first 3 days), 50 to 100 mg. daily, divided as above.

AVAILABLE on prescription through all pharmacies in original bottles of 100 and 1,000 scored tablets (50 mg. each).



HEDULIN is not cumulative in effect — provides greater uniformity of action and ease of maintenance.

HEDULIN is rapidly excreted — therapeutic effect dissipated within 24-48 hours, if withdrawal becomes necessary.

HEDULIN acts promptly — producing therapeutic prothrombin levels in 18-24 hours.

HEDULIN requires fewer prothrombin determinations—only one every 7-14 days after maintenance dose is established.

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Clinical experience shows that the best diet for reducing is a normal diet modified by reduction in portion size of certain foods rather than by their elimination. Desirable and necessary foods in the "ideal reducing diet" include "meat, poultry, fish, eggs, milk and other dairy products, leafy green and yellow vegetables, citrus fruits, and enriched and whole grain products."*

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*Berryman, G. H.: Obesity—A Brief Review of the Problem, Metabolism *3*:544 (Nov.) 1954.

The nutritional statements in this advertisement have been reviewed by the Council on Foods and Nutrition of the American Medical Association and found consistent with current authoritative medical opinion.

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1. Shackman, N. H., Heffer, E. T., and Kroop, I. G.: Am. Heart J. 48:599 (Oct.) 1954. • 2. Stollerman, G. H., and others: Am. J. Med. 15:645 (Nov.) 1953. • 3. McEwen, C.: M. Clin. North America 39:353 (March) 1955. • 4. Wood, H. F., and McCarty, M.: Am. J. Med. 17:768 (Dec.) 1954. • 5. McEwen, C., and Ziff, M.: M. Clin. North America (May) 1955, to be published.

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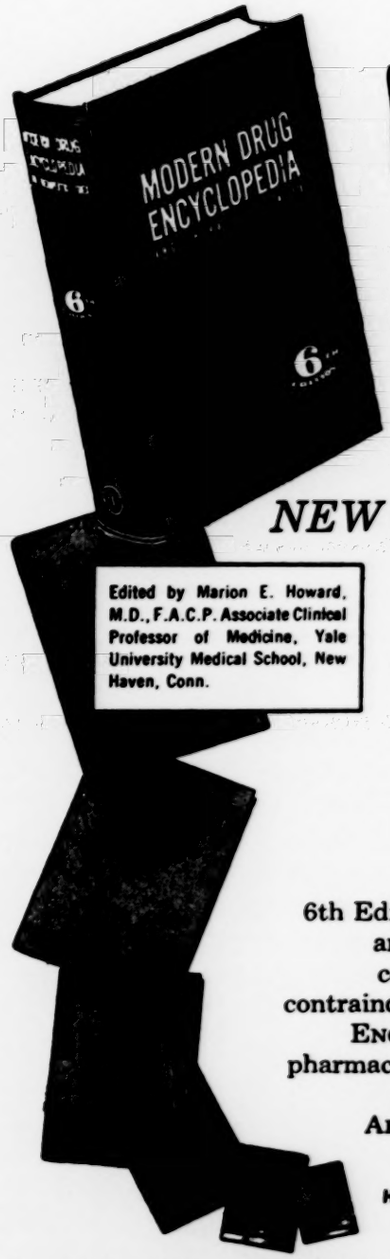

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